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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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The Kinetics of Degradation of Chloramphenicol in Solution

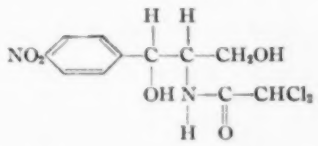
I. A Study of the Rate of Formation of Chloride Ion in Aqueous Media*

By T. HIGUCHI and CHARLES D. BIAS†

A chemical kinetics study of the rate of formation of chloride ions in aqueous solutions of chloramphenicol at elevated temperatures has been made. The data obtained indicate that the over-all rate of chloride production is a summation of the rates of at least three first-order reactions: direct uncatalyzed hydrolytic cleavage of chlorine ions from whole chloramphenicol molecules, direct hydroxyl ion catalyzed cleavage of chloride ions from whole chloramphenicol molecules, and hydrolysis of chloramphenicol at the amide linkage, then subsequent hydrolysis of the dichloroacetate ions formed to yield chloride ions and other degradation products. The rates of the several reactions have been separated by mathematical analysis from each other in the over-all rate and the heats of activation have been determined. The results for the dichloroacetate reaction are in excellent agreement with those of Kunze. The present study is an example of how kinetic studies can be used to elucidate the mechanism of breakdown of complex drugs.

CHLORAMPHENICOL is believed to be the most chemically stable of the antibiotics in common use. It has been reported (1) that no significant degradation occurs after boiling for five hours with distilled water or after standing for twenty-four hours at a *p*H range from 2 to 9. On prolonged standing, however, it does undergo significant degradation, with consequent loss of activity. Other than the above observations, there has been no considered kinetic study made of the degradative reaction.

Because of the multiplicity of functional groups in chloramphenicol, the degradative mechanism could be extremely complicated. From the formula (2) it will be seen that degradation may occur by hydrolysis of the amide, by chloride hydrolysis, by oxidation to the ketone or aldehyde, by reduction of the nitro group, etc. Various



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† This paper is based in part on a thesis submitted by C. D. Bias to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Master of Science.

C. D. Bias is Parke, Davis & Co. Fellow, University of Wisconsin.

The authors of this paper would like to express their appreciation to Parke, Davis and Co. for their sympathetic cooperation and assistance on this study. They would like in particular to acknowledge the help given by George Moersch, Albert L. Rawlins, J. M. Vandenbelt, and Larry Wheeler.

combinations of these reactions may occur simultaneously or consecutively. Any study of the degradative processes of such a compound would involve not only qualitative identification of the various reactions which the separate functional groups undergo, but also the orders and rates of the individual reactions.

The present paper is concerned with the chemical kinetics of those processes yielding chloride ions from chloramphenicol, and, as such, is a part of a broad program on the elucidation of the over-all degradative pathways of this important antibiotic. Because of its relatively high stability at room temperature, chloramphenicol lends itself very nicely to such a kinetic study. Chloride ion hydrolysis seemed a good starting point, first, because of the ease and simplicity of such a study, and secondly, because it was felt that it might possibly be the most important degradative process occurring.

Since chloramphenicol is soluble in water only to the extent of about 0.5 per cent, or less than 0.02 molar, at the temperatures of study, chloride ion concentration during hydrolysis would be rather small, especially during the early phases of the reaction. If 100 ml. of 0.5 per cent chloramphenicol were used, and 5-ml. samples withdrawn, each sample would, on complete degradation, contain approximately only 0.20 millimole of chloride ions. In order to follow the chloride ion formation when as little as one part in a thousand of the drug had degraded, a method was needed which would be accurate down to 0.0002 millimole of chloride ion. Amperometric titration with silver nitrate has been used successfully with fairly minute quantities of chloride ions (3). By using a microburet with 0.005 *N* silver nitrate, and a fine delivery tip calibrated to give 0.01 ml. per drop, a sensitivity of 0.00005 millimole could be obtained, thus making it possible to follow chloride ion formation to within 0.04 per cent of the total available halide.

EXPERIMENTAL

Reagents Used.—Phosphate-sodium hydroxide buffers, ranging in *p*H from 5.8 to 8.0, in increments of 0.2 *p*H units; 0.005 *N* alcoholic silver nitrate solution; saturated ammonium nitrate solution; acetone; nitric acid; and 1% gelatin solution.

Procedure Used for Determination of *p*H and Temperature Effect.—Exactly 0.5000 Gm. of chloramphenicol, which had previously been recrystallized from ethanol and ethyl acetate, was weighed into each of twelve 100-ml. volumetric flasks. To each of these flasks was added 95 ml. of buffer solution ranging in *p*H from 5.8 to 8.0, in increments of 0.2 *p*H units. The flasks were then placed in a constant temperature bath set at 87.0°. After several minutes' lapse to allow the solutions to equilibrate thermally, the solutions were brought to the mark with additional buffer solutions. Periodically, 5-ml. samples were pipetted from each flask into 30-ml. beakers. The first sample was withdrawn in three hours, to give an indication of the initial rate, and a total of 6 samples was withdrawn over a period of fifty hours.

The withdrawn samples were then analyzed for their ionic chloride content. For this, 10 ml. of acetone (to decrease the water concentration and thus decrease silver chloride going into solution), 1 ml. of a saturated solution of ammonium nitrate (to

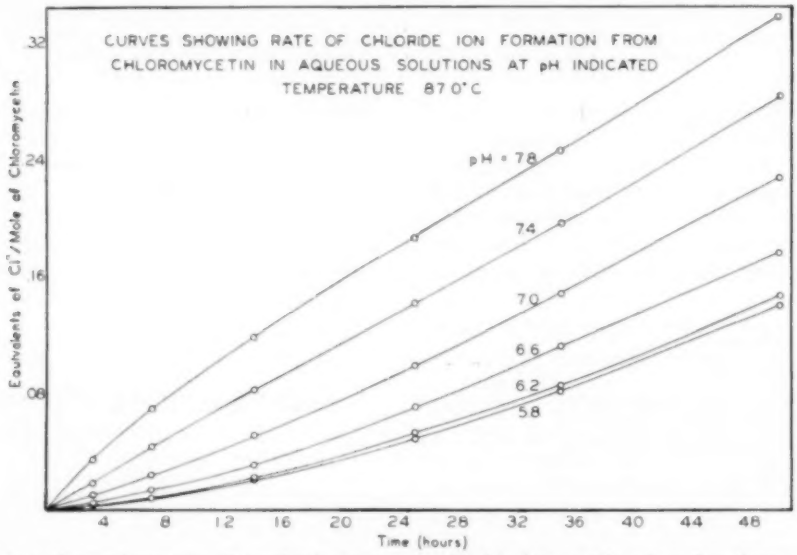


Fig. 1.—Plots of experimentally determined amount of chloride ions produced as functions of time in several aqueous solutions buffered to different *p*H values. These runs were made at 87.0° C.

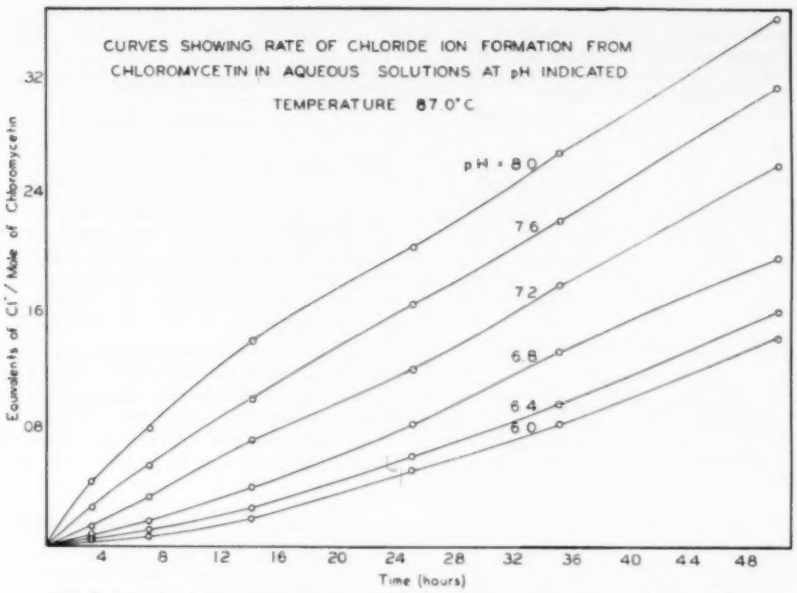


Fig. 2.—Runs similar to those shown in Fig. 1, except at different *p*H values.

furnish sufficient electrolyte for the passage of current), 1 drop of a 1% gelatin solution (to coat the silver chloride particles and prevent possible reduction of the silver ion at the electrode), and 1 drop of concentrated nitric acid (since it was found that at higher *p*H values, silver oxide was formed, thus making it impossible to detect the true end point) were added to the withdrawn aliquot samples. The resulting mixtures were then titrated amperometrically, with a rotating platinum electrode and with 0.005 *N* alcoholic silver nitrate solution.

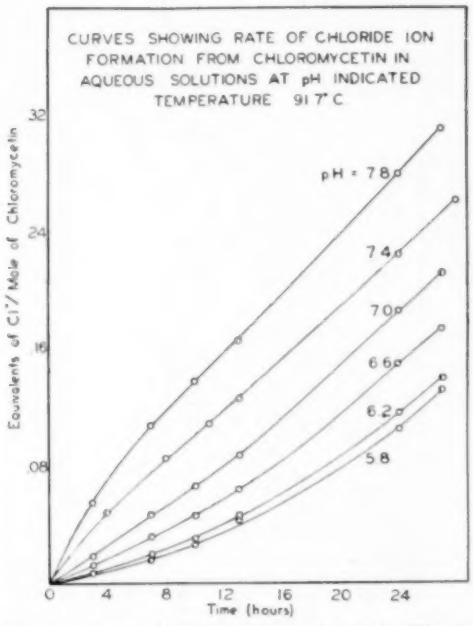


Fig. 3.—Runs similar to those shown in Fig. 1, except at 91.7°.

The above procedure was repeated, changing only the temperatures used, 91.7° and 97.0°, and the times of withdrawal to allow for the increased rate of hydrolysis.

Initially, duplicate samples were withdrawn from the bath and titrated, but later the high degree of precision attained permitted the discontinuance of the duplicate samples.

The equivalents of chloride ion found per mole of chloramphenicol were calculated in the following manner:

$$\frac{(\text{ml. } AgNO_3)(N\ AgNO_3)(0.32314)}{0.025} = \text{equiv. of } Cl^-$$

where 0.32314 is the milliequivalent weight of chloramphenicol and 0.025 is the weight of chloramphenicol contained originally in 5 ml.

Procedure for Determination of the Order of the Reaction with Respect to Chloramphenicol.—Essentially the same procedure as above was used except for the necessary changes in the concentration of the drug. Six runs were made at *p*H 5.8 and 7.6 with concentration varying from 0.1 to 0.6% in 0.1% steps. The temperature was held at 97.0° for this entire study.

RESULTS AND DISCUSSION

pH and Temperature Dependencies of the Reaction.—Plots of the equivalents of chloride ion found per mole of original chloramphenicol against the time that the samples had been withdrawn from the bath are shown for each *p*H solution and for each temperature used in Figs. 1–6. The curves obtained do not, at first glance, seem to fit any particular pattern, and by their complexity indicate that

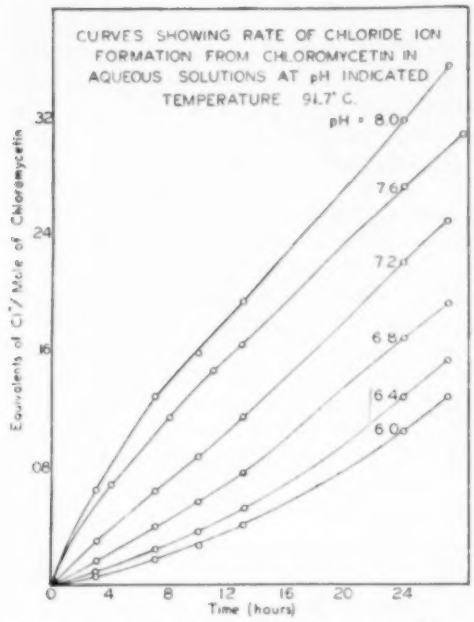
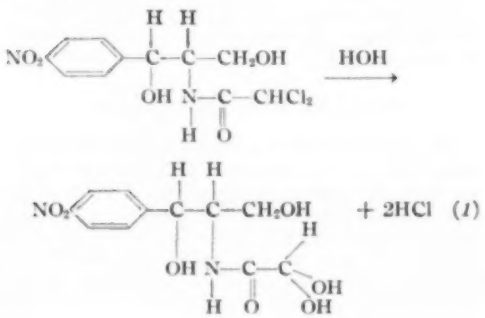


Fig. 4.—Runs similar to those shown in Fig. 3, except at different *p*H values.

the over-all rate of chloride ion formation is probably a summation of rates of a series of reactions. The problem of disentangling the several reactions involved proved, however, to be not too difficult.

It can be seen from the curves (Figs. 1–6) that the *initial* rates of chloride ion formation increase gradually as the *p*H increase. These initial rates, or more specifically, equivalents of chloride ion per hour initially per mole of chloramphenicol, were calculated from the graphs by drawing tangents to the curves as near the origin as feasible. The results are shown in Figs. 7, 8, and 9, where equivalents of chloride ion per hour per mole were plotted against *p*H for each of the three temperatures. It will be observed that at the lowest *p*H values the rates are the same, but increase, at first gradually and then rapidly, as the *p*H is increased. It is evident that the initial stage of chloride ion production is composed of at least two reactions, namely a *p*H-independent reaction and a *p*H-dependent reaction. For these reactions which are responsible for chloride ion production during the initial degradative stage, we can write the following reactions:



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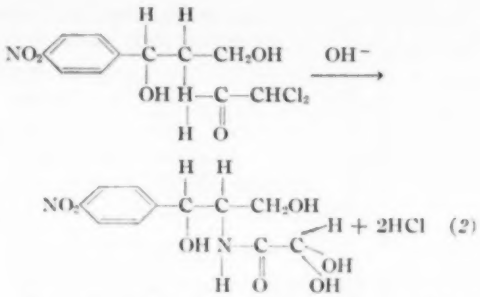


Separation of these reactions can be accomplished mathematically by subtracting the rates at the lower *p*H values (the *p*H-independent reaction *1*) from the rates at higher *p*H values. The actual numerical values of the rate constant of the *p*H-independent reaction responsible for chloride pro-

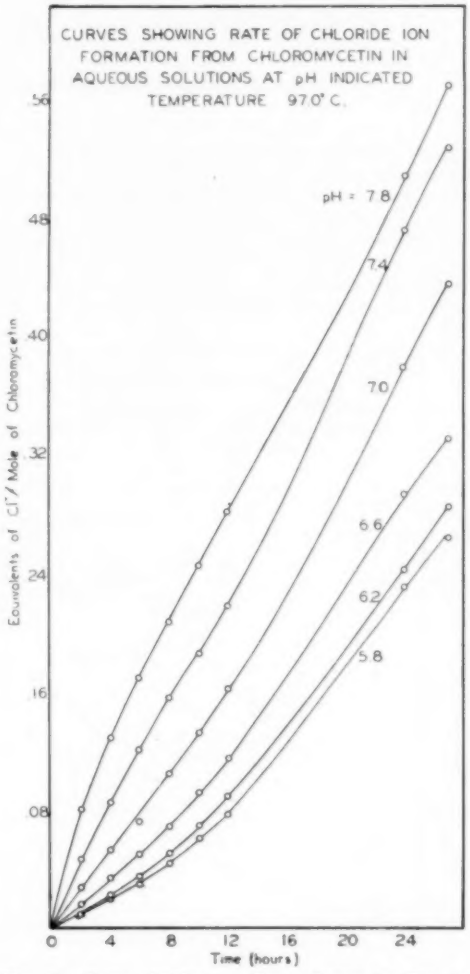


Fig. 5.—Runs similar to those shown in Fig. 1 except that these were run at 97.0°.

duction during the initial period as estimated from the experimental data are shown in Table I for determinations at 87.0, 91.7, and 97.0°. Any increase in the rate above these values which may result from an increase in *p*H can be attributed directly to the *p*H-dependent reaction. The degree of dependency of the rate of this second reaction on *p*H is shown in Fig. 10, where the logarithms of the rates of this reaction obtained by subtracting the rates of the first reaction from the observed over-all rates have been plotted against *p*H. It can be seen that the resulting functions are essentially straight lines having slopes of 1, indicating that the rate of the second reaction is of the first order with respect to hydroxide ion concentration. It will be observed that the values at higher *p*H values do not fall on the lines. This is attributed, at least in part, to the difficulty in determining the initial reaction rates at higher *p*H values from the graphs, since in these cases the initial rates were quite rapid and highly time-dependent.

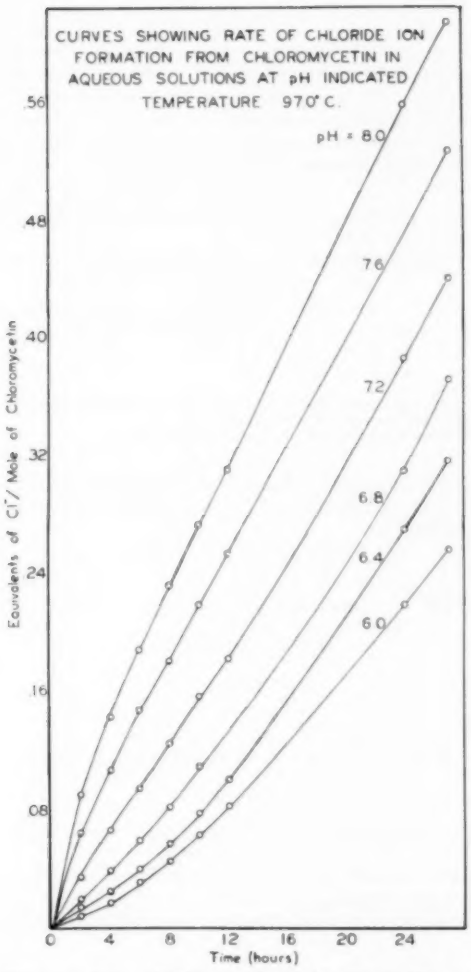


Fig. 6.—Runs similar to those shown in Fig. 5, except at different *p*H values.

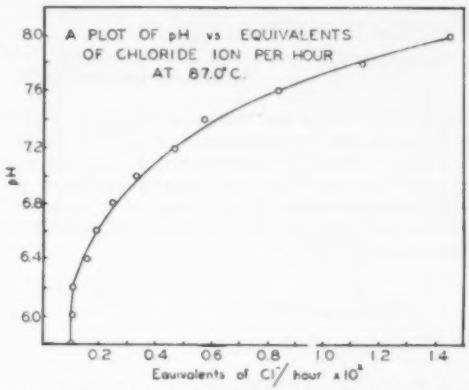


Fig. 7.—A plot showing the influence of *p*H on the *initial* rate of chloride production in solutions of chloramphenicol kept at 87.0° C. The independence of the rate of *p*H at lower *p*H values is very apparent from this curve.

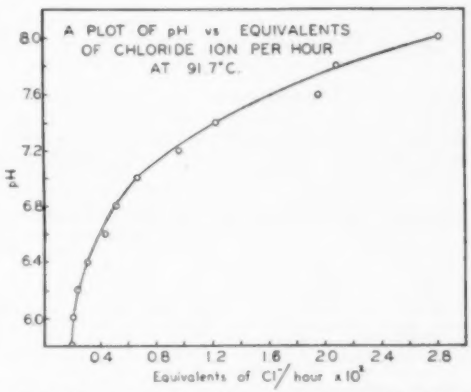


Fig. 8.—A plot showing the influence of *p*H on the *initial* rate of chloride production in solutions of chloramphenicol kept at 91.7° C.

TABLE I.—EVALUATION OF THE RATE CONSTANT OF THE *p*H-INDEPENDENT REACTION RESPONSIBLE FOR CHLORIDE PRODUCTION DURING THE INITIAL STAGE[a]

	Observed Rates Expressed as Moles Cl^- Produced/Hr./Mole Chloramphenicol		
*p*H	87.0° C.	91.7° C.	97.0° C.
5.8	0.0010	0.0020	0.0038
6.0	0.0010	0.0020	0.0038
6.2	0.0010	0.0024	0.0050
Estimated k_1 =	0.0010	0.0020	0.0038

[a] The limiting values at the two lower *p*H runs were used to estimate the rate constant of the *p*H independent reaction. Since two chloride ions are produced from each molecule of the drug, the actual rate constant of Reaction *1* would be one half of these values.

On the basis of the above considerations it is possible to relate the rate of chloride production by reaction *2* to the over-all rate of chloride formation and to derive the specific rate constant for this reaction in terms of *p*H and the observed rate. It is evident that

$$\text{Rate of } Cl^- \text{ formation by reaction 2} = \frac{d(Cl^-)}{dt} - k_1\,(\text{Chloramphenicol}) = k_2\,(\text{Chloramphenicol})(OH^-)$$

where $d(Cl^-)/dt$ is the total observed rate of chloride production in equivalents of chloride formed per hour. Since the experimental initial rates were recorded as equivalents of chloride produced per mole of chloramphenicol per hour, it is more convenient to divide the above expression through by the drug concentration;

$$\frac{d(Cl^-)}{(\text{Chloramphenicol})\,dt} - k_1 = k_2(OH^-).$$

And solving for k_2, we find,

$$k_2 = \frac{1}{(OH^-)}\left(\frac{d(Cl^-)}{(\text{Chloramphenicol})\,dt} - k_1\right) = \frac{(H^+)}{K_w}\left(\frac{d(Cl^-)}{(\text{Chloramphenicol})\,dt} - k_1\right)$$

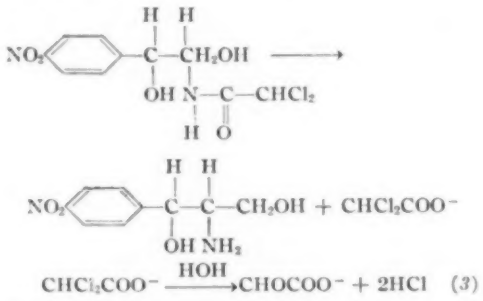
or

$$\log k_2 = pK_w - p\text{H} + \log\left(\frac{d(Cl^-)}{(\text{Chloramphenicol})\,dt} - k_1\right)$$

As would be expected log k_2 comes out as a linear function of pH. Numerical values for k_2 can be determined by substitutions into the above equation. In Table II the rate constant so obtained has been calculated for several temperatures from data determined at pH 6.80, 7.00, and 7.20, these being the values which fell on the straight line portion of the plots shown in Fig. 10. The pK_w values at these temperatures were interpolated from the data of Harned and Hammer (4).

Heats of Activation of Reactions 1 and 2.—The heats of activation of both reactions can be readily determined from the data in Table I and Table II in the usual way by plotting the logarithms of the rate constants against the reciprocals of the corresponding absolute temperatures. This has been done in Fig. 11 for the pH-independent reaction (the line marked "initial") and in Fig. 12 for the pH-dependent reaction. On the basis of the slopes of these lines the heat of activation for reaction *1* is calculated to be approximately 35.4 kilocalories and that for reaction *2* to be 26.1 kilocalories. If the heat of ionization of water is added to the latter value, an apparent heat of activation of 35.3 kilocalories is obtained for the second reaction.[1]

Consideration of the Over-all Reaction After the Initial Stage.—A further observation may be made from the graphs (Figs. 1–6) during the later course of the reaction. It will be seen that the limiting rates at all pH values are almost identical since the slopes toward the end of each curve are about the same. This is considered to be a pH-independent reaction which cannot occur initially, but is dependent on building up a sufficient concentration of an intermediate reactant which itself is formed by an hydrolytic reaction. These experimental observations are compatible with the following suggested reaction mechanism:



Chloramphenicol undergoes initially a hydrolysis reaction yielding an amine and a dichloroacetate ion. Subsequently, dichloroacetate ions, which are grad-

TABLE II.—EVALUATION OF THE RATE CONSTANT OF THE pH-DEPENDENT REACTION RESPONSIBLE FOR CHLORIDE PRODUCTION DURING THE INITIAL STAGE

	87.0° C.		91.7° C.		97.0° C.	
pH	Net Rate[a]	k_2[b]	Net Rate[a]	k_2[b]	Net Rate[a]	k_2[b]
6.80	0.0015	6.55×10^2	0.0032	11.2×10^2	0.0059	18.2×10^2
7.00	0.0023	6.34	0.0047	10.8	0.0085	16.6
7.20	0.0037	6.43	0.0077	11.2	0.0140	17.2
	Average	6.44		11.1		17.3

[a] The net rates attributable to the pH-dependent reaction were obtained by subtracting k_1 values given in Table I from the observed initial rates expressed in moles of chloride produced per hour per mole of chloramphenicol.
[b] The values were obtained by dividing the corresponding net rates by the hydroxyl ion concentrations present.

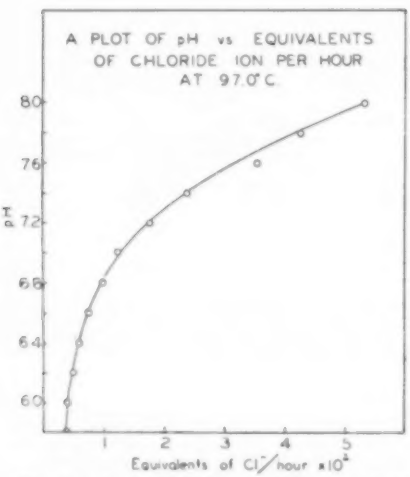


Fig. 9.—A plot showing the influence of pH on the *initial* rate of chloride production in solutions of chloramphenicol kept at 97.0° C.

[1] See reference (5) for comments on the relationship of the heat of ionization of water to heats of activation of hydroxide catalyzed reactions.

ually building up in concentration, undergo further hydrolysis giving off chloride ions in a *p*H-independent reaction. That this hydrolysis of dichloroacetate ion is *p*H-independent has been established by Kunze (6) who found that hydroxyl ion accelerated the reaction only to the extent to which it neutralizes the hydrochloric acid which originates in the reaction or to the extent it increases the degree of dissociation of dichloroacetic acid through the formation of sodium salts.

If we accept the above mechanism as being essentially correct, it is possible to compare directly the data obtained in this study with those of Kunze. If we assume that by the time the limiting slope is attained essentially all of chloramphenicol has been converted to dichloroacetate, the slopes themselves are then equal to the rate constant. The rate constants so calculated are given in Table III where the apparent limiting rate of chloride formation expressed in equivalents of chloride formed per hour per mole of original chloramphenicol is given for several temperatures and *p*H values. The specific rate constant of reaction *3*, is actually one half of this since two chloride ions are produced from a molecule of the drug.

TABLE III.—EFFECT OF *p*H ON THE LIMITING RATE OF PRODUCTION OF CHLORIDE IONS FROM CHLORAMPHENICOL

*p*H	Rates in Equiv. of Cl^- Produced/Hr./Mole Chloramphenicol 87.0° C.	91.7° C.	97.0° C.
7.2	0.0055	0.010	0.018
7.4	0.0058	0.009	0.019
7.6	0.0061	0.009	...
7.8	0.0061	0.010	0.019
8.0	0.0062	0.012	0.018
Average	0.0059	0.010	0.0185

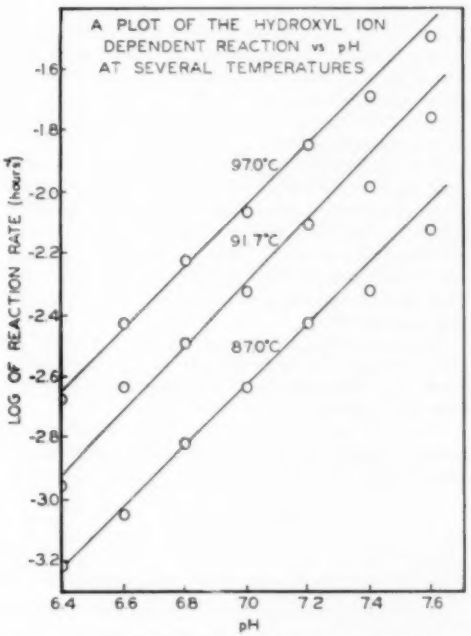


Fig. 10.—Plots showing the first order dependency of the *p*H-dependent reaction on hydroxyl ion concentration at several temperatures. All three lines were drawn with fixed slopes of 1.00. It is evident that the theoretically predicted slope gives excellent fit in all three cases.

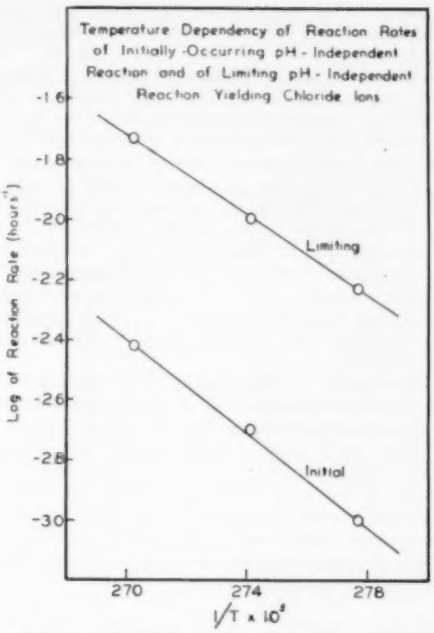


Fig. 11.—Arrhenius type plots of the two *p*H independent reactions responsible for chloride formation from chloramphenicol. The lower plot is that of the initially dominant reaction. The upper plot is that of the limiting reaction which appears to operate on some breakdown product of the antibiotic.

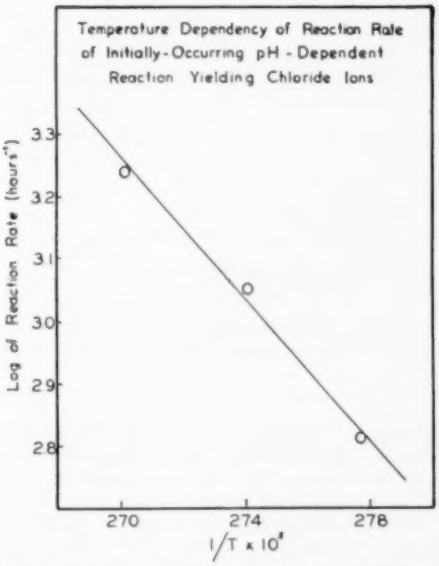


Fig. 12.—Arrhenius type plot of the *p*H dependent reaction shown in Fig. 10.

Kunze in his study of the hydrolytic rate of dichloroacetate ion obtained specific reaction rate constants of 0.0575 hr.$^{-1}$ at 114.5° and 0.0131 hr.$^{-1}$ for 100° for this reaction. These are in close agreement with the corresponding values of 0.060 hr.$^{-1}$ and 0.0135 hr.$^{-1}$ obtained by extrapolation of the data in Table III and dividing by two. The data in Table III, when plotted in the usual way for determination of heat of activation as shown in Fig. 11 (curve marked "limiting"), yield a straight line corresponding to activation energy of 30.6 kilocalories. In comparison Kunze obtained a value of 31.4 for dichloroacetate. In view of these agreements it appears probable that the limiting rate of chloride production from chloramphenicol occurs substantially through the dichloroacetate mechanism as given above.

Dependency of the Over-all Rate on Chloramphenicol Concentration.—So far in the consideration of the several reactions involved in the production of chloride ions from chloramphenicol, it has been assumed that the reactions were of the first order with respect to the drug. Although this seemed to be a fairly safe assumption since all of the reactions involved were simple hydrolytic molecular reactions, it was thought desirable, nevertheless, to establish this point experimentally.

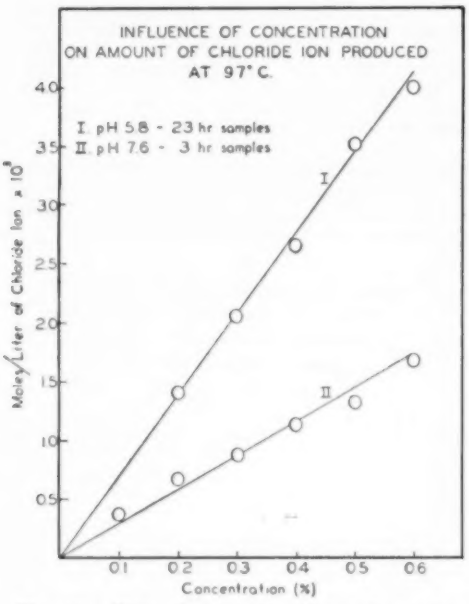


Fig. 13.—Plots showing the first order nature of the chloride formation with respect to the drug itself.

In Fig. 13 the concentrations of chloride ion found after being held at 97° for twenty-three hours at *p*H of 5.8 have been plotted against the original concentration of chloramphenicol in per cent. A similar plot of another series of runs made at a higher *p*H but for a shorter time is also shown. It is evident from both plots that the amount of chloride ion produced is substantially a first order function of the substrate concentration.

The runs at the higher *p*H showed greater variations. There were indications that the glass walls of the reaction vessels were attacked in degrees corresponding somewhat to the concentration of the drug present.

Over-all Reaction in Terms of the Individual Reactions.—The over-all reaction of chloramphenicol in water to yield chloride ions together with other degradation products can be fairly well synthesized from the three chemical reactions discussed. During the initial phase of the reaction chloride ion production is solely due to direct hydrolysis of chloramphenicol as caused by reaction *1* and reaction *2*. At a *p*H of 6 or lower the uncatalyzed reaction *1* is principally responsible for the degradative action. Above this *p*H the catalytic action of hydroxyl ion as shown in the second equation becomes increasingly important. Concurrently with these reactions another type of hydrolytic cleavage is occurring at the amide linkage which is not reflected directly by chloride ion determination. Like all hydrolytic reactions of amides and esters, this reaction may be expected to be of the first order with respect to hydroxyl ion concentration. The reaction itself does not yield chloride ions but it influences the over-all rate of their production in two ways: first, by decreasing the substrate for reactions *1* and *2*, and secondly, by yielding dichloroacetate ions which break down into chloride ions and other degradation products. The first effect is shown in the manner with which the rate of chloride ion production decreases sharply after an initial spurt in high *p*H systems. The second effect is noticeable in runs made at relatively low *p*H since the uncatalyzed hydrolytic rate of dichloroacetate ion is much greater than that of unchanged chloramphenicol, leading to a progressively increasing rate of chloride ion production. These expectations are in accord with experimental facts as given in Figs. 1–6.

It is highly probable that additional reactions, aside from those discussed here, are also responsible for degradation of chloramphenicol. It is doubtful, however, that they can change the over-all picture presented to any great extent. Oxidation or internal oxidation-reduction reactions may occur, but they, in all probability, do not affect the rate of chloride production.

Further studies are in progress to determine exactly whether the three postulated reactions can account for substantially all of chloramphenicol lost from the system.

This investigation serves to illustrate the value of kinetic studies in solving complex reactions responsible for drug breakdown.

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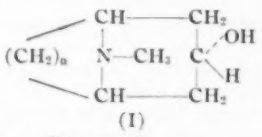
Stereoisomeric N-Methylgranatolines*

By WALTER H. HARTUNG† and S. M. GADEKAR‡

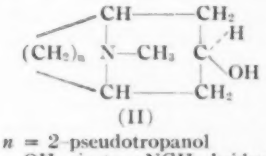
From a comparison of the physical properties, *p*H values, and on the basis of chemical genesis it is suggested that the N-methylgranatoline obtained by the reduction of pseudopelletierine with sodium and alcohol is sterically related to pseudotropanol. The isomeric N-methylgranatoline obtained by catalytic hydrogenation is placed in the same steric configuration as tropanol.

PSEUDOPELLETIERINE, also known as N-methylgranatonine is the chief alkaloid of pomegranate bark (1). Its structural similarity to tropanone was established by Willstätter (2) and by Piccinini (3). It has been synthesized under so-called "biological" conditions from glutaraldehyde, methylamine, and acetone dicarboxylic acid by Menzies and Robinson (4), by Schöpf and Lehmann (5), and by Cope and coworkers (6). N-methylgranatoline, the carbinol obtained by reducing the ketone, N-methylgranatonine, is of interest because of its homology to tropanol, and like tropanol it is optically inactive and nonresolvable, and yet capable of existing in two stereoisomeric structures, I and II.



(I)

n = 2-tropanol
—OH *trans* to :NCH_3 bridge
and *cis* to ethylene bridge
n = 3-"isomethylgranatoline"



(II)

n = 2-pseudotropanol
—OH *cis* to :NCH_3 bridge
and *trans* to ethylene bridge
n = 3-"methylgranatoline"

For the tropanol isomers the configuration has been established, that corresponding to I as, tropanol, the amino alcohol obtained by the hydrolysis of atropine; and the configuration of II is assigned to pseudotropanol, which is obtained by the hydrolysis of tropacocaine (7). For the medicinal chemist such stereoisomerism would appear to be very significant. Liebermann and Limpbach (8) quote Professor O. Liebreich as stating that the tropic acid ester of pseudotropanol "wirkt. . . .nicht als Mydriaticum," although in other respects it is reported to exhibit the physiological properties of atropine, but with half the potency.

The isomeric N-methylgranatolines are also known. Ciamician and Silber reduced pseudopelletierine by means of sodium and alcohol and called their product "methylgranatoline." Werner (9) employed concentrated hydriodic acid and zinc dust to obtain the other isomer, which he called "isomethylgranatoline." Werner also examined the mandelic and tropic acid esters of the two isomeric N-methylgranatolines and reported that the esters of methylgranatoline were strongly mydriatic, whereas the esters of isomethylgranatoline were devoid of mydriatic properties. Werner did not indicate any steric relationship of the N-methylgranatolines to the isomeric tropanols.

We have prepared pseudopelletierine and reduced it in two ways, just as was done for the preparation of the two tropanols; the reduction of tropanone with sodium and alcohol gave pseudotropanol; and catalytic reduction with platinum oxide gave tropanol (10). On the basis of evidence now available it appears that the reduction methods are stereo-specific, that with sodium and alcohol the newly formed hydroxyl group takes a position *cis* to the N-methyl bridge and when formed by catalytic hydrogenation the –OH group is *trans* to the N-methyl bridge.

We also prepared the mandelic esters of the two isomeric N-methylgranatolines for study of their mydriatic properties. Our findings differ from those reported by Werner (9) in that both showed mydriatic activity, the ester obtained via the sodium-ethanol route probably being slightly less active. These results suggest that the physiological activity of the respective esters would not be a safe criterion for putting methylgranatoline in the same configurational series with tropanol. Chemical genesis of the isomers and a consideration of their physical properties appear as a more reliable guide; these assign to isomethylgranatoline the configuration shown in I.

Smith (*7a*), in assigning configuration to the tropanol, reasoned from a study of models that the structure corresponding to II may be expected to show steric hindrance with respect to both the

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TABLE I.—COMPARISON OF ISOMERIC TROPANOLS WITH ISOMERIC N-METHYLGRANATOLINES

	Obtained by Reducing with Na + EtOH		Obtained by Reducing Catalytically (PtO_2)	
	Methylgranatoline	Tropanol	Methylgranatoline	Tropanol
Free base, m. p.	100°	108° (12)	65° (9)	63° (11)
Picrate, m. p.	249 (4)	258–259° (12)	263–264 (1)	275° (11)
Mandelic ester as base	Noncrystalline (12)	Noncrystalline (11, 12)	.....	95.5–98.5 (11)
Mandelic ester hydrobromide	Hydroscopic syrup	[a]	229° (9)	217–218 (11)
Tropic ester base	Noncrystalline	86–88° (11)	Noncrystalline (9)	118° (13)
Tropic ester hydrobromide	220 (9)	[b]	233 (9)	163–164 (13)

[a] The hydrochloride is hygroscopic and resinifies (8); sulfate, hygroscopic.
[b] Hydrochloride melts with decomposition at 183°.

amino and carbinol functions, and that there might be evidence of bonding between the two. Esterification rates and *p*K and *p*H values support these assumptions. An analogous condition is seen to exist for the N-methylgranatolines. The differential *p*K and *p*H values, though not so marked as for the tropanol isomers, are in harmony with the hypothesis that catalytic hydrogenation gives rise to configuration I and sodium-ethanol reduction forms II.

The results of our mydriatic tests, though of an exploratory character, also raise the question about the validity of the reported nonmydriatic activity of pseudotropanol esters. It would be most remarkable to find that the presence of an extra methylene grouping in II, that is where $n = 3$, should convert the compound from a nonmydriatic to a mydriatic precursor. Perhaps the esters of pseudotropanol merit reinvestigation.

EXPERIMENTAL

Pseudopelletierine.—This was prepared according to the procedure described by Cope and co-workers (6).

Hydrogenations.—These were carried out as described by Keagle and Hartung for the preparation of the tropanol isomers (10). The product resulting from the catalytic hydrogenation of pseudopelletierine formed a colorless distillate, b. 139°, under a pressure of 11–12 mm., which solidified to a translucent, hygroscopic solid, m. 69°; picrate (formed instantly), m. 263–264°. These values agree with those reported by Werner (9) for isomethylgranatoline. The product obtained by sodium-alcohol reduction distilled 145–148° under a pressure of 14 mm., formed a waxy solid, m. 96°; yielded a picrate (formed slowly) m. 249°. These values agreed with those reported for methylgranatoline.

Mandelic Esters.—One Gm. methylgranatoline and 1 Gm. mandelic acid were dissolved in just enough cold 10% HCl to effect complete solution, and refluxed for seventy-two hours. The solution was cooled and extracted with ether to remove excess mandelic acid, then made basic, and again extracted with ether to remove mandelic ester. The ether extracts were dried over anhydrous sodium sulfate and treated with an ethereal solution of HBr.

TABLE II.—TITRATION OF 20.0 ML. OF 0.05 *N* N METHYLGRANATOLINE WITH 0.0495 *N* HCl

Acid Added, Ml.	*p*H as Titration Proceeds I	II
0.0	11.82	11.69
1.0	11.62	11.52
4.0	11.21	11.02
8.0	10.62	10.51
12.0	10.27	10.17
16.0	9.42	9.40
20.0	7.01	7.03
20.5	5.81	5.95
20.7	4.91	5.00
20.9	3.25	3.41
21.5	2.27	2.37
22.0	1.92	2.05

The hydrobromide of the ester prepared from I, after drying over P_2O_5 and two crystallizations from absolute ethanol, melted 227°; reported m. p. 229° (9). The HBr salt of the other isomer, prepared similarly, when dried over P_2O_5 formed a brownish solid which was difficult to purify, even after three crystallizations from alcohol-ether. Werner (9) described this salt as a viscous syrup.

Mydriatic Activity.[1]—These tests were made by exposing one eye of the rabbit to the compound under observation and the other to atropine sulfate. The results indicate that both mandelic esters are less active than atropine and that the isomer derived from structure I is more active.

Titration.—Twenty milliliters of a 0.05 *N* solution of each N-methylgranatoline isomer was diluted with 30.0 ml. water and titrated with 0.0495 *N* HCl. The *p*H readings were made after each addition of acid. Part of the data are given in Table II. Although the differences are not large, the results are consistent and suggest a hindrance or probably, hydrogen bonding (7c) in that isomer assigned structure II.

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[1] We are grateful to the late Professor C. W. Chapman for assistance and guidance in these tests.

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Antispasmodics. V. Miscellaneous Tertiary Aminoalkyl Esters*

By ROBERT BRUCE MOFFETT† and CHARLOTTE ANNE HART

IN CONCLUDING the work on antispasmodics in these laboratories a number of new basic esters have been prepared (Table III). Except as otherwise noted these compounds were prepared by the Method *A* described in the third article of this series (1). The necessary intermediate acids (Table II) and malonic esters (Table I) were made by methods which have been illustrated in previous work (1–4), or are described in the Experimental part.

The basic esters were tested on isolated muscle but none of them had antispasmodic activity in the useful range.

EXPERIMENTAL

Cyclopentyl-(β-diethylaminoethyl)-malonic acid hydrochloride.—A mixture of 50 Gm. (0.153 mole) of diethyl cyclopentyl-(β-diethylaminoethyl)-malonate with a saturated solution of sodium hydroxide in 100 ml. of 95% ethanol was heated in a bomb immersed in an oil bath at 140–150° for three hours. The contents of the bomb were dissolved in water, filtered, extracted with ether, made strongly acidic with hydrochloric acid, and again extracted with ether. The aqueous solution was distilled to dryness *in vacuo* on a steam bath. The crystalline residue was refluxed with 500 ml. of absolute ethanol, filtered, and the filtrate was distilled to dryness. This was again taken up in 200 ml. of absolute ethanol, filtered, and cooled in the refrigerator. Crystals separated and were collected and dried; weight, 20.3 Gm. (37.6%); m. p. 133–134° (dec.).

Anal.—Calcd. for $C_{14}H_{25}NO_4 \cdot HCl.C_2H_5OH$: Cl, 10.02. Found: Cl 10.21.

Cyclopentyl-(β-diethylaminoethyl)-acetic acid.—The alcoholic filtrate from the above preparation was evaporated to dryness and combined with 17.5 Gm. of the malonic acid. This was heated in an oil bath at 165–170° under an atmosphere of nitrogen until no more gas was evolved. The resulting glassy solid was dissolved in water, extracted with ether, filtered, and shaken for one and one-half hours with a suspension of silver oxide prepared from 38.4 Gm. of silver nitrate. The mixture was filtered, extracted with ether, saturated with hydrogen sulfide, and again filtered. The filtrate was distilled to dryness under reduced pressure on a steam bath. The residue was dissolved in acetone, treated with decolorizing charcoal, and filtered, giving a clear, nearly colorless solution which, on concentrating to about 100 ml. and cooling, deposited crystals The product was collected, washed with acetone, and dried, giving 25.5 Gm. (77.5%, based on the malonic ester) of white crystals, m. p. 63–67°.

Anal.—Calcd. for $C_{13}H_{25}NO_2$: N, 6.16. Found: N, 6.17.

Δ²-Cyclopentenyl-(β-diethylaminoethyl)-malonic acid hydrochloride.—In a way similar to that described above, this compound was obtained from 50 Gm. (0.154 mole) of diethyl Δ²-cyclopentenyl-(β-diethylaminoethyl)-malonate. Yield, 28.1 Gm. (60%); m. p. 157–158° (decomp.).

Anal.—Calcd. for $C_{14}H_{23}NO_4 \cdot HCl$: Cl, 11.60. Found: Cl, 11.68.

Δ²-Cyclopentenyl-(β-diethylaminoethyl)-acetic acid.—In a way similar to that described above, 23.6 Gm. of the above malonic acid hydrochloride and the residue from the evaporation of the filtrate from its crystallization were decarboxylated and worked up, giving 17.2 Gm. (54.5%, based on the malonic ester) of white crystals, m. p. 69–75°.

Anal.—Calcd. for $C_{13}H_{23}NO_2$: N, 6.22. Found: N, 6.30.

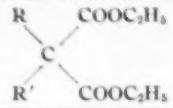
Ethyl 4-benzoxycyclohexyl-cyanoacetate.—This was prepared from 4-benzoxycyclohexanone and ethyl cyanoacetate by the method Alexander and Cope (6) used for ethyl cyclohexylcyanoacetate. The yield was 84%; b. p. 174° (0.027 mm.); n_D^{25} 1.5132.

Anal.—Calcd. for $C_{15}H_{21}NO_4$: N, 4.44. Found: N, 4.29.

Ethyl (4-benzoxycyclohexyl)-ethyl-cyanoacetate.—To a suspension of 5.3 Gm. (0.23 mole) of powdered sodium in 30 ml. of dry toluene was slowly added with stirring a solution of 65 Gm.

* Received April 3, 1953, from the Research Laboratories of George A. Breon and Company.
The functions of the George A. Breon and Company Laboratories have been assumed by the Sterling-Winthrop Research Institute, Rensselaer, N. Y., and any requests for reprints should be addressed there.
† Present address: The Upjohn Company, Kalamazoo, Mich.

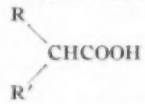
TABLE I.—MALONIC ESTERS[a]



R[b]	R'[b]	Yield, %	B. p. °C.	B. p. Mm.	n_D^{25}	d_4^{25}	Formula	Molecular Refractivity Calcd.	Molecular Refractivity Found
Cyclopentyl	β-Diethylaminoethyl[c]	98.4	91	0.01	1.4604	0.9991	$C_{18}H_{32}NO_4$	89.56	89.84
Δ¹-Cyclopentenyl	β-Diethylaminoethyl[d]	57.6	110	0.1	1.4658	1.0088	$C_{18}H_{31}NO_4$	89.09	89.31
Δ¹-Cyclohexenyl	1-Acenaphthyl[e]	52.4	180	0.005	...	...	$C_{25}H_{28}O_4$	...	...
Hydrogen	1-Hydrindenyl	56.7	112	0.03	1.5025	1.0963	$C_{16}H_{20}O_4$	73.61	74.34
1-Hydrindenyl	Allyl	72.5	122	0.04	1.5100	1.0821	$C_{19}H_{24}O_4$	87.00	87.45
1-Hydrindenyl	Benzyl[f]	73.5	158	0.03	1.5420	1.1160	$C_{23}H_{26}O_4$	102.34	103.33
Phenyl	1-Acenaphthyl[g]	54.6	165	0.005	...	...	$C_{28}H_{24}O_4$	...	...
Tetrahydrofurfuryl	Benzyl[h]	80.2	128	0.01	1.4988	1.0982	$C_{19}H_{26}O_5$	89.11	89.37
Tetrahydrofurfuryl	Cyclohexylmethyl[h]	73.0	105	0.02	1.4700	1.0511	$C_{19}H_{32}O_5$	90.52	90.37

[a] Most of these malonic esters were not fully characterized by elementary analyses, but were converted to solid barbituric acids for this purpose. These are reported in the accompanying paper together with other barbituric acids.
[b] In each case, R is the first group introduced into the malonic ester, and R' is the second group.
[c] *Anal.* Calcd.: N, 4.28. Found: 4.06.
[d] *Anal.* Calcd.: N, 4.30. Found: 4.26.
[e] On standing, this ester crystallized, and a sample was recrystallized from petroleum hexane; m. p. 78.5–80°.
[f] On standing, it crystallized, and a sample was recrystallized from ethanol; m. p. 49–51°.
[g] A sample was recrystallized from petroleum hexane; m. p. 89–91°.
[h] Prepared by alkylating in the usual way diethyl tetrahydrofurfurylmalonate (8).

TABLE II.—SUBSTITUTED ACETIC ACIDS



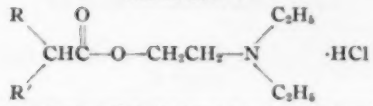
R	R'	Yield, %	B. p. °C.	B. p. Mm.	n_D^{25}	d_4^{25}	Formula	Molecular Refractivity Calcd.	Molecular Refractivity Found	Neutral Equivalent Calcd.	Neutral Equivalent Found
1-Hydrindenyl	Allyl	...	128	0.045	1.5392	1.0816	$C_{14}H_{16}O_2$	62.13	62.67	216.3	218.5
1-Hydrindenyl	Benzyl	51.4[a]	165	0.045	...	...	$C_{18}H_{18}O_2$	...	...	266.3	265.9
Phenyl	1-Acenaphthyl	36.6[b]	...	...	...	...	$C_{20}H_{16}O_2$	...	...	288.3	287.8 289.1
Tetrahydrofurfuryl	Benzyl	83.5	147	0.055	1.5252	1.1159	$C_{14}H_{18}O_3$	64.24	64.36	234.3	238.7
Tetrahydrofurfuryl	Cyclohexylmethyl[c]	91.0	135	0.04	1.4836	1.0436	$C_{14}H_{24}O_2$	65.65	65.83	240.3	246.3
Cyclopentyl	β-Diethylaminoethyl[d]	77.5									
Δ²-Cyclopentenyl	β-Diethylaminoethyl[d]	54.5									

[a] Yield of material recrystallized from methanol, m. p. 148–152°.
[b] Yield of material recrystallized from acetone, m. p. 212–216°.
[c] On standing, it crystallized, and a sample was recrystallized from petroleum hexane, m. p. 73–76°.
[d] See experimental part for preparation and properties.

(0.205 mole) of ethyl 4-benzoxycyclohexyl-cyanoacetate in 100 ml. of dry toluene. The mixture was refluxed for one hour, during which the sodio-derivative separated as a gummy precipitate. To this was added 38 Gm. (0.35 mole) of ethyl bromide. After refluxing for two and one-half hours, the mixture tested nearly neutral. A few drops of acetic acid and a little water were added, the layers were separated, and the aqueous layer was extracted with ether which was added to the ester and washed with dilute sodium bicarbonate and then with saturated salt solution. After removal of the solvent, the product was distilled from a Claisen flask, b. p. 185° (0.1 mm.), giving 42.5 Gm. (60.5%) of a viscous liquid; n_D^{25} 1.5106.

In an attempt to remove any mono-substituted cyanoacetic ester which might be present, the product was shaken at room temperature for seven hours with 100 ml. of 20% aqueous sodium hydroxide. It was diluted with water, taken up in ether, well

TABLE III.—



R	R′	Yield, %[a]	M. P., °C.	Analyses N, % Calcd.	N, % Found	Cl, % Calcd.	Cl, % Found
Cyclopentyl	β-Diethylaminoethyl	94.0[b]	156.5–158.5	...	...	17.75	17.72
Δ²-Cyclopentenyl	β-Diethylaminoethyl	42.6[c]	166–170	...	...	17.84	17.46
1-Hydrindenyl	Allyl	56.0	72.5–76	3.98	3.81	10.07	10.02
1-Hydrindenyl	Benzyl	80.0	96–105	3.49	3.45	8.84	8.86
Phenyl	1-Acenaphthyl	58.0	176.5–179	3.31	3.40	8.36	8.39
Tetrahydrofurfuryl	Benzyl	60.0	77–86	3.79	3.81	9.59	9.83
Tetrahydrofurfuryl	Cyclohexylmethyl	60.0	109–112.5	3.73	3.69	9.43	9.51
Phenyl	1-Piperidyl	...[d]	187–189	...	...	18.14	18.16
Phenyl	Bromo	26.[e]	122–126	...	...	45.96[f]	46.2[f]
Cyclopentyl	β-Cyclohexylethyl	66.0	153–154.5	3.61	3.70	9.14	9.24
R CH is R′	2-Phenylcyclohexyl[g]	43.5	63–73	...	...	10.45	10.40

[a] The yield is based on the substituted acetic acid (Table II). The free bases were not characterized unless indicated.
[b] This yield is based on the free base which was isolated in 58% yield from the corresponding acid; b. p. 94° (0.02 mm.); n_D^{25} 1.4628; d_4^{25} 0.9260. *Anal.* Calcd. for $C_{19}H_{30}N_2O_2$: N, 8.58. Found: 8.50.
[c] This yield is based on the free base which was isolated in 43% yield, recrystallized from ethanol plus ethyl acetate, from the corresponding acid; b. p. 97° (0.01 mm.); n_D^{25} 1.4680; d_4^{25} 0.9355. *Anal.* Calcd. for $C_{19}H_{34}N_2O_2$: N, 8.64. Found: 8.04.
[d] This was prepared by a modification of the method of Burtner and Cusic (5) as described in the experimental part.
[e] This was prepared from the corresponding acid chloride by Method *B* described in our previous work (1).
[f] Total halogen (% chlorine + % bromine).
[g] This was prepared by Mr. James E. Stafford in these laboratories from 2-phenylcyclohexylcarboxylic acid by the method of Burtner and Cusic (5). The hydrochloride was recrystallized from ethyl acetate.

washed with water and saturated salt solution, and dried over sodium sulfate. Redistillation gave 30 Gm of light yellow viscous liquid; n_D^{25} 1.5098.

Anal.—Calcd. for $C_{20}H_{32}NO_4$: N, 4.08. Found: N, 3.86.

On long standing, crystals separated which were collected and well washed with cyclohexane which removed the residual oil. The yield of white crystals was 4.2 Gm., m. p. 92–93°. The nitrogen analysis was the same on the crystals as on the oil. It is therefore presumed that the crystals represent one of the *cis-trans* modifications.

β-Diethylaminoethyl α-(1-piperidyl)-phenylacetate.—To a solution of the acid, obtained by the hydrolysis of 10 Gm. (0.04 mole) of ethyl α-(1-piperidyl)-phenylacetate (7), in 50 ml. of isopropanol, was added 5.4 Gm. (0.04 mole) of β-diethylaminoethyl chloride. After refluxing for five hours, the reaction mixture was made basic with sodium ethoxide and diluted with ether. This was filtered from the salt and dried over sodium sulfate. The solvent was removed and the free base was distilled under reduced pressure, giving 5 Gm. (40%) of liquid; b. p. 65° (1 mm.); n_D^{25} 1.5070; d_4^{25} 1.0184.

The hydrochloride was precipitated by hydrogen chloride from a solution of the free base in absolute ether. This was recrystallized from methyl isobutyl ketone, giving a white crystalline product (Table III).

SUMMARY

1. A number of new esters of tertiary amino alcohols have been prepared.

2. Intermediate malonic esters and substituted acetic acids are reported.

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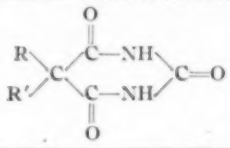
Barbituric Acids*

By ROBERT BRUCE MOFFETT,† JAMES E. STAFFORD,† and CHARLOTTE ANNE HART

In connection with other work in these laboratories (1–5) a large number of malonic esters were available, most of which had not been previously described. In order to characterize them further, it was thought desirable to prepare solid derivatives. We have, therefore, made a series of barbituric acids from most of those malonic esters by allowing them to react with urea in the presence of sodium ethoxide or sodium methoxide.

Table I records the physical properties of these barbituric acids. They were all prepared by

Table I.—Barbituric Acids



R	R′	Yield, %[a]	M. p., °C.	Empirical Formula	Analysis, % Nitrogen Calcd.	Found
n-Amyl	Δ²-Cyclopentenyl	21.	120–122[d]	$C_{14}H_{20}N_2O_3$	10.60	10.31
iso-Amyl	Δ²-Cyclopentenyl	4.5	128–133[d]	$C_{14}H_{20}N_2O_3$	10.60	10.71
2-Methylbutyl	Δ²-Cyclopentenyl	29.2	125–131[e]	$C_{14}H_{20}N_2O_3$	10.60	10.56
2-Ethylbutyl	Δ²-Cyclopentenyl	9.2	146–149	$C_{15}H_{22}N_2O_3$	10.07	9.83[b]
2-Chloroallyl	Δ²-Cyclopentenyl	9.5	183–186	$C_{12}H_{13}ClN_2O_3$	10.39	10.43
Benzyl	Δ²-Cyclopentenyl	46.8	219–221	$C_{16}H_{16}N_2O_3$	9.86	9.93
2-Cyclohexylethyl	Δ²-Cyclopentenyl	55.8	175–180	$C_{17}H_{24}N_2O_3$	9.21	9.33
2-Phenoxyethyl	Δ²-Cyclopentenyl	62.3	246–248	$C_{17}H_{18}N_2O_4$	8.92	8.96
Δ²-Cyclohexenyl	Δ²-Cyclopentenyl	24.6	183–184	$C_{16}H_{18}N_2O_3$	10.22	10.46
Cyclohexylmethyl	Δ²-Cyclopentenyl	33.7	212–214	$C_{16}H_{22}N_2O_3$	9.65	9.52
Furfuryl	Δ²-Cyclopentenyl	32.	171–173.5	$C_{14}H_{14}N_2O_4$	9.65	9.76
Cyclopentyl	Δ²-Cyclopentenyl	44.	162–165.5[f]	$C_{14}H_{18}N_2O_3$	10.69	10.15
2-Thienylmethyl	Δ²-Cyclopentenyl	41.4	208.5–209.5	$C_{14}H_{14}N_2O_3S$	9.65	9.37
2-Diethylaminoethyl	Δ²-Cyclopentenyl	44.	175–177[d]	$C_{15}H_{23}N_3O_3$	14.33	14.24
n-Propyl	Cyclopentyl	68.4	177–178	$C_{12}H_{18}N_2O_3$	11.76	11.60
n-Butyl	Cyclopentyl	36.	172–174	$C_{13}H_{20}N_2O_3$	11.11	11.18
iso-Butyl	Cyclopentyl	30.	168–172	$C_{13}H_{20}N_2O_3$	11.11	10.86
n-Amyl	Cyclopentyl	27.	[d]	$C_{14}H_{22}N_2O_3$	10.52	10.44
iso-Amyl	Cyclopentyl	15.	145–147	$C_{14}H_{22}N_2O_3$	10.52	10.23
2-Methylbutyl	Cyclopentyl	12.	131–135	$C_{14}H_{22}N_2O_3$	10.52	10.35
2-Ethylbutyl	Cyclopentyl	5.	171–173[d]	$C_{15}H_{22}N_2O_3$	9.99	9.74
2-Cyclohexylethyl	Cyclopentyl	48.3	164–166.5	$C_{17}H_{22}N_2O_3$	9.15	9.31
Benzyl	Cyclopentyl	54.	245–247	$C_{14}H_{18}N_2O_3$	10.68	10.81
Furfuryl	Cyclopentyl	43.2	190–191.5	$C_{14}H_{16}N_2O_4$	9.61	9.63
2-Thienylmethyl	Cyclopentyl	43.7	219–220	$C_{14}H_{16}N_2O_3S$	9.58	9.56
2-Diethylaminoethyl	Cyclopentyl	53.7	159–160[d]	$C_{15}H_{25}N_3O_3$	14.23	14.14
n-Propyl	Δ²-Cyclohexenyl	37.	146–148	$C_{13}H_{18}N_2O_3$	11.19	11.45
n-Butyl	Δ²-Cyclohexenyl	39.5	160–163	$C_{14}H_{20}N_2O_3$	10.59	10.71
2-Methylbutyl	Δ²-Cyclohexenyl	14.	160–162[d]	$C_{15}H_{22}N_2O_3$	10.07	9.93
2-Ethylbutyl	Δ²-Cyclohexenyl	19.6	178–179	$C_{16}H_{24}N_2O_3$	9.58	9.37
Δ²-Cyclohexenyl	Δ²-Cyclohexenyl	25.	202–213	$C_{16}H_{20}N_2O_3$	9.71	9.69
Cyclohexylmethyl	Δ²-Cyclohexenyl	35.	229–231	$C_{17}H_{24}N_2O_3$	9.21	9.25
2-Cyclohexylethyl	Δ²-Cyclohexenyl	51.8	189–193	$C_{18}H_{26}N_2O_3$	8.80	8.81
Benzyl	Δ²-Cyclohexenyl	44.1	234–239	$C_{17}H_{18}N_2O_3$	9.39	9.45
Furfuryl	Δ²-Cyclohexenyl	52.5	219–221	$C_{15}H_{16}N_2O_4$	9.21	9.34
Cyclohexyl	2-Cyclohexylethyl	34.4	210–212	$C_{18}H_{28}N_2O_3$	8.74	8.72
Cyclohexyl	Benzyl	36.7	267–269	$C_{17}H_{20}N_2O_3$	9.33	9.09
1-Hydrindenyl	Allyl	63.	168–170	$C_{16}H_{16}N_2O_3$	9.86	9.90
1-Hydrindenyl	Benzyl	31.	196–199	$C_{20}H_{18}N_2O_3$	8.38	8.34
Tetrahydrofurfuryl	Benzyl	81.6[c]	239–241	$C_{16}H_{18}N_2O_4$	9.27	9.45
Tetrahydrofurfuryl	Cyclohexylmethyl	63.	192–194	$C_{16}H_{24}N_2O_4$	9.09	9.08

[a] The yields are reported for recrystallized products. In most cases, no attempt was made to work up the filtrates.
[b] Chlorine analysis—Calcd.: Cl, 13.20. Found: Cl, 13.18.
[c] This yield is based on unrecrystallized material, m. p. 237–239°.
[d] Crystallized from 95% ethanol.
[e] Crystallized from benzene plus petroleum hexane.
[f] Crystallized from benzene.

* Received April 3, 1953, from the Research Laboratories of George A. Breon and Company.
The functions of the George A. Breon and Company laboratories have been assumed by the Sterling-Winthrop Research Institute, Rensselaer, N. Y., and any requests for reprints should be addressed there.
† Present address: The Upjohn Company, Kalamazoo, Mich.

essentially the same general method which is given in the Experimental part.

EXPERIMENTAL

General Method for the Preparation of Barbituric Acids.—A mixture of 0.1 mole of the malonic ester, 0.22 mole of sodium ethoxide (or sodium methoxide), 0.13 mole of urea, and 65 ml. of absolute ethanol was refluxed for about six hours. The solvent was then removed by distillation and the residue was heated in an oil bath at 110–120° for two hours. After cooling, the mixture was thoroughly shaken with water, extracted twice with ether, and acidified with acetic acid. The barbituric acids usually separated as oils which crystallized on standing and scratching. The solid was collected on a filter, dried, and recrystallized, usually from methanol, adding a little water if necessary to secure good separation.

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The Development of Organic Iodine Compounds as X-Ray Contrast Media*

By V. H. WALLINGFORD†

A FEW YEARS AGO Rigler (1) of the University of Minnesota stated that "approximately 80 per cent of patients in hospitals and 70 per cent of those seen in outpatient clinics will be submitted to some form of roentgen study at some period in the course of their illness." That statement serves to point out the important position X-ray diagnosis has attained in modern medical practice. X-ray contrast media, including the organic iodine compounds discussed in the present paper, have played an essential role in this development.

Immediately following the discovery of X-rays in 1895 scientists attempted to use this new tool to explore the interior of the living human body, and to view structures otherwise visible only during surgery or autopsy. While the bones were clearly visualized by X-rays, other parts or organs of the body were not distinguishable because they lacked contrast with the surrounding tissues. Some visualization was attained by surrounding the soft tissues under study with dense materials such as lead shot or iron powder. Cannon (2) of the Harvard Medical School, a pioneer radiologist in this country, studied the esophagus of the goose, using a paste of bismuth salts as the contrasting agent. To hold the subject stationary enough for the comparatively long exposures required by the X-ray tubes of that day, he enclosed the neck of the goose in cardboard formed like a mailing tube. Bismuth subnitrate came into general use in 1904 (3) for the examination of the gastrointestinal tract, only to be supplanted in 1910 (4) by barium sulfate. The Mallinckrodt Chemical Works entered the field of X-ray contrast media in 1913 with the introduction of a grade of barium sulfate specially prepared for this application.

The element iodine has been found particularly useful for incorporation into X-ray contrast media. It exhibits high absorption of X-rays in the range or wave lengths applicable to the human body. Sodium iodide has long been used for cystography and even for intravenous injection. Because of its toxic side reactions and its irritant effect on mucous surfaces, however, it has been largely displaced by the organic iodine compounds. The chemical nature of iodine permits its combination into stable, nontoxic organic substances specially adapted to specific techniques of X-ray diagnosis.

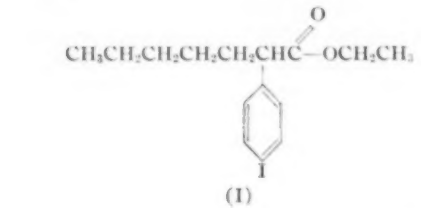
IODIZED OILS

The first organic iodine compound to be used as an X-ray contrast medium was Lipiodol, introduced in 1922 (5). Chemically, this is poppy seed oil containing organically bound iodine. In the intervening years a number of iodized oils have been prepared from various unsaturated vegetable oils

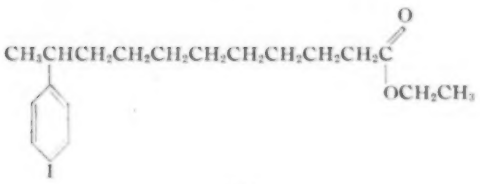
* Received August 20, 1953, from the Research Laboratories of the Mallinckrodt Chemical Works, St. Louis, Mo.
Presented to the Scientific Section of the A. PH. A., Salt Lake City meeting, August 19, 1953, by V. H. Wallingford, recipient of the Fifth (1952) Chilean Iodine Educational Bureau Award for outstanding research in the chemistry and pharmacology of iodine.
† This work on iodine compounds has been shared by many individuals. To all of them I express my appreciation. Special thanks are due to Dr. Melvin A. Thorpe who, from the beginning, has shared my hopes and enthusiasm and has spared no effort in arranging clinical trials and caring for the many details necessary for the introduction of a new medicinal.

For example, Iodochloral (Searle) is peanut oil containing iodine and chlorine. The introduction of these iodized oils paved the way for many new radiological techniques. The iodized oils are used in more areas of the body than any other medium. They are the media of choice for visualizing the bronchial tree (bronchography), the spinal column (myelography), the sinus tracts, and the uterus and oviducts (uterosalpingography). They also find use for delineating such structures as the lacrimal ducts, seminal vesicles, and various fistulae. The iodized natural oils are bland and nonirritating. They are slowly adsorbed and the iodine is liberated as inorganic iodide. Figure 2 shows a typical bronchogram made with an iodized oil.

Our laboratory prepared a number of iodized oils by adding iodine monochloride or hydrogen iodide to unsaturated oils. This was a tricky problem because the original oils, as well as the products containing iodine, were readily subject to autoxidation and polymerization. Many storage tests were made, some extending for five years or more. Although some of these oils were doubtless satisfactory for radiological use, we were not satisfied with the reproducibility of our procedure and hesitated to offer them for clinical use. We became intrigued by the possibility of attaching iodine to an aromatic nucleus in which it would be firmly held. Judicious tasting of the esters on the laboratory shelves showed that blandness increased with the length of the carbon chain containing the carboxylic acid. Following this lead we prepared a number of esters of iodinated phenylalkanoic acids of which compound I, ethyl α-(p-iodophenyl)-heptanoate is typical.



(I)

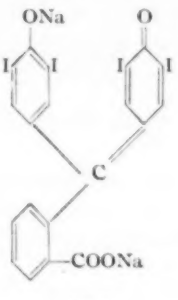


(II)

Although satisfactory with respect to iodine content and viscosity, these esters proved to be irritating to the bronchial mucosa in dogs and were discarded. Our next plan was to move the phenyl group bearing the iodine atom further from the carboxyl group, but the synthetic problems seemed considerable and the project was delayed. Had we carried out this plan we doubtless would have obtained useful compounds, for not long thereafter Pantopaque (II) was introduced as a useful iodized oil. In this substance the phenyl group bearing an iodine is near the end of a long alkyl chain.

CHOLECYSTOGRAPHY

The early radiological procedures involved the direct placement of the opaque medium into the site to be examined by X-rays. In 1909 Abel and Rowntree (6) observed that tetrachlorophenolphthalein was excreted by the liver and concentrated in the gall bladder. In 1923 Graham (7) of the Washington University School of Medicine (St. Louis, Mo.) conceived the brilliant idea of substituting bromine or iodine in place of the chlorine in tetrachlorophenolphthalein with the expectation that the dye would be excreted by the liver and that the heavier bromine or iodine atoms would absorb the X-rays and produce a shadow of the gall bladder. In cooperation with the Mallinckrodt Laboratories, sodium tetraiodophenolphthalein (Iodeikon) (III) was made available in 1924. This product enjoyed acceptance as the standard contrast medium for visualizing the gall bladder for twenty years.



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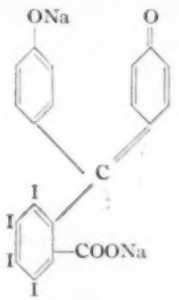


(IV)

Iodeikon is administered orally or intravenously and later becomes concentrated in the gall bladder. This radiological technique, called cholecystography, represented the first practical use of a natural physiological process for collecting a contrast medium in the desired region.

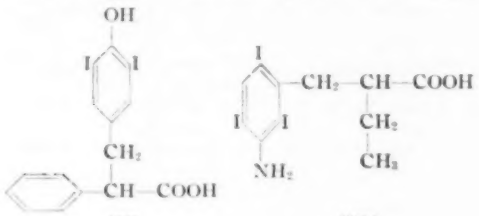
Considerable effort was expended in attempts to find a medium superior to tetraiodophenolphthalein. In 1928 (8) Graham and his associates published a list of 46 compounds that had been tested up to that time. Many more were tested later. The exploration of this field was my own introduction to X-ray contrast media. However, nothing superior to Iodeikon and its closely related Iso-Iodeikon was found for many years. The latter substance, phenoltetraiodophthalein (IV), has certain advantages in that its concentration in the blood can readily be determined colorimetrically. The rate of disappearance of the dye from the blood stream has been used as a measure of liver function.

The development of cholecystography is notable for a number of reasons. The original conception by Graham is a good example of the "flash of genius" by which many important advances are made. Unquestionably this pioneering development stimulated and inspired later work in the field of intravenous contrast media. It is also an excellent example of fruitful cooperation between an industrial laboratory and an academic institution. Such cooperation was not a very common occurrence in those days.

My own experience, gained by improving the processes for Iodeikon and Iso-Iodeikon and in

synthesizing other organic iodine compounds for testing in cholecystography, prepared me for a later cooperative research with Copher and his associates, also at Washington University. My part in this program was to prepare tetraiodophenolphthalein containing radioactive iodine (I^{131}) for use in the direct irradiation of carcinoma of the liver and biliary tract. With the help of borrowed instruments for measuring radiation a safe procedure was improvised. To save time, in view of the short half-life of I^{131} (eight days), and to avoid risk to other personnel, all important operations were carried out at night. Over 25 separate syntheses were thus performed without at any time subjecting myself to more than 40% of the permissible daily irradiation. The results of this investigation, published in 1952 (9) show that the radiation, resulting from the disintegration of artificially radioactive tetraiodophenolphthalein is dissipated in the gall bladder and surrounding liver tissue. The intensity of radiation attainable by this means, however, was judged insufficient to be therapeutically effective in the gall bladder although it might possibly be effective in the liver tissue itself.

Further developments in the field of cholecystography were hampered by the lack of precise information on the factors governing the absorption of a substance from the gastrointestinal tract and its concentration in the gall bladder. In 1940 (10) there was synthesized in Germany a new cholecystographic agent, β-(4-hydroxy-3,5-diiodophenyl)-α-phenylpropionic acid (Biselectan) (V), which was later introduced into this country by the Schering Corporation as Priodax. This has become an important cholecystographic medium. A cholecystogram of a normal human gall bladder made with Priodax is shown in Fig. 3. Other recently developed cholecystographic agents are Telepaque (VI) (Winthrop-Stearns), Monophen (VII) (National Synthetics), and Teridax (VIII) (Schering).



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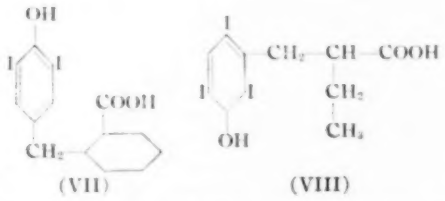
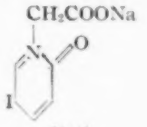


(VII) (VIII)

Space does not permit a discussion of the extensive and excellent chemical work that resulted in the development of these contrast agents. Examination of the formulas of the useful cholecystographic agents reveals something of a pattern. They all contain an aromatic ring bearing the iodine separated from a carboxyl group by an organic group, the nature of which varies from case to case.

UROGRAPHY

Our most recent activities and contributions have been in the field of urography, the X-ray examination of the urinary system. Some of the earlier contrast media used for this purpose were sodium iodide, iodized oils, and suspensions of bismuth salts or barium sulfate. These substances were introduced in the desired region by the retrograde method, that is, by means of a catheter. Following the pioneering work of Graham and Cole and of Rowntree in utilizing a natural physiological function to collect and concentrate an X-ray medium, there was developed in Germany in 1928 (11) a substance called Uroselectan (IX). This was the first urographic medium designed for intravenous use. During the next few years there was introduced a number of pyelographic agents which are still accepted media for this purpose: Skiodan (X) and Diodrast (XI) (Winthrop-Stearns), Neo-Iopax (XII) (Schering Corp.), and Hippuran (XIII) (Mallinckrodt). Urokon (XIV), the most recent pyelographic contrast medium, was made available by Mallinckrodt in 1951 (12, 13).



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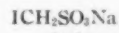

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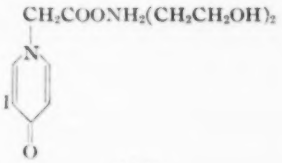

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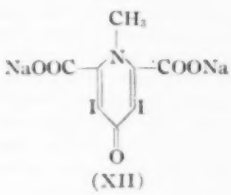

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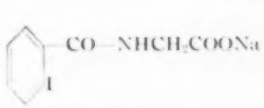

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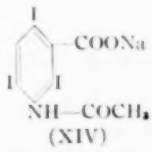


(XIV)

Hippuran was introduced in 1933 by Swick (14), who had been associated with the development of the original urographic medium Uroselectan. The conception of Hippuran was based on the fact that hippuric acid is frequently present in normal urine and is the form in which the human body disposes of the more toxic benzoic acid. It seemed logical, therefore, that hippuric acid containing iodine would be relatively nontoxic and also would be excreted through the urinary system. These hopes were realized in the sodium salt of ortho-iodo-hippuric acid (Hippuran). This product serves admirably

in retrograde pyelography but because of its lower iodine content gives shadows less dense than those produced by the polyiodo compounds when administered intravenously.

In an attempt to improve upon Hippuran, Paul A. Krueger, then in my laboratory, synthesized a number of analogous compounds. The benzene ring portion was altered by changing the position of the iodine atom and by introducing more than one iodine. Glutamic acid was also used in place of the aminoacetic acid which appears in Hippuran. Table II lists the compounds that were prepared.

TABLE I.—HIPPURAN ANALOGS

	Solubility, Gm. Na Salt/100 Cc.	M. T. D.,[a] Gm./Kg.
o-Iodohippuric acid (Hippuran)	100	3.1
m-Iodohippuric acid	60	1.5
p-Iodohippuric acid	80	1.3
2,5-Diiodohippuric acid	17	1.2
3,4-Diiodohippuric acid	45	0.5
2,3,5-Triiodohippuric acid	3	...
2-Iodo-3,5-dibromohippuric acid	1.4	...
N-(*o*-Iodobenzoyl)-glutamic acid	67	2.0
N-(2,5-Diiodobenzoyl)-glutamic acid	>70	2.6
p-Iodophenaceturic acid	67	1.1

[a] M. T. D. is the highest dose at which 3 out of 5 rats lived. Values are somewhat lower than the true LD_{50}.

Two of these compounds were studied by Brewer (15) at the University of Michigan. When injected intravenously into rabbits, 2,5-diiodohippuric acid (as the triethanolamine salt) was poorly excreted and was definitely more toxic than Hippuran. Sodium N-(2,5-diiodobenzoyl)glutamate was tolerated in about the same doses as Hippuran but was excreted in a slightly more concentrated form in the urine. We were disappointed that none of the products showed properties significantly superior to *ortho*-iodohippuric acid. Klemme and Hunter (16) of Purdue University have also explored this series, apparently with no greater success.

THE DEVELOPMENT OF UROKON

As in cholecystography the search for more suitable urographic media was hampered by the lack of guiding principles concerning the relationship of chemical structure to such properties as solubility, toxicity, and mode of elimination from the human body. The problem is difficult, because the desired compound must not only be opaque to X-rays and have the property of being selectively excreted but it should also cause a minimum of side reactions, such as nausea, dizziness, allergic manifestations, and the like. In 1944 I chanced to read an article in *Science* on a totally unrelated subject, the excretion of penicillin, and came upon the statement that *para*-aminohippuric acid, given intravenously to mice, was surprisingly nontoxic. Here was a compound that should be easily iodinated. I could visualize derivatives with the amino group in other positions and analogs with the aminoacetic acid portion replaced by other amino acids such as we had prepared in the Hippuran problem. I undertook the problem myself during spare moments since I felt that to direct research properly, one should maintain first hand familiarity with organic reactions. The literature disclosed no case of an iodinated aminohippuric acid or related compound. One conceivable synthesis of compounds of the hippuric acid type would be the acetylation of an aminoiodobenzoic acid, conversion to the acid chloride, and condensation with the desired amino acid. This method of synthesis proved futile, but fortunately I had resolved to examine all of the intermediates in order to detect trends in toxicity and solubility which might point to useful compounds. We were surprised to find that the introduction of an acetyl group markedly altered the properties of the parent aminoiodobenzoic acid. A similar change in properties was caused by many other acyl groups. The comparisons are shown in Table II.

TABLE II.—COMPARISON OF AMINOIODOBENZOIC ACIDS AND THEIR ACYL DERIVATIVES

Benzoic Acid Derivative	Solubility of Sodium Salt, Gm./100 Cc.	LD_{50}, Mg./Kg.
2-Amino-3,5-diiodo-	13.3	180
3-Amino-2,4,6-triiodo-	9.3	1,450
4-Amino-3,5-diiodo-	2.56	515
2-Acetylamino-3,5-diiodo-	38.8	1,530
2-Butyrylamino-3,5-diiodo-	...	560
2-(*n*-Caproylamino)-3,5-diiodo-	105.0	365
2-Benzoylamino-3,5-diiodo-	...	360
2-(*α*-Phenylbutyrylamino)-3,5-diiodo-	...	385
2-(*o*-Iodobenzoylamino)-3,5-diiodo-	...	575
3-Formylamino-2,4,6-triiodo-	70.5	8,000
3-Acetylamino-2,4,6-triiodo-	94.2	9,560
3-Propionylamino-2,4,6-triiodo-	37.2	8,100
3-Butyrylamino-2,4,6-triiodo-	59.3	4,800
3-Benzoylamino-2,4,6-triiodo-	15.9	1,000
3-Isobutyrylamino-2,4,6-triiodo-	29.3	3,700
3-Caproylamino-2,4,6-triiodo-	77.6	1,450
3-Caprylylamino-2,4,6-triiodo-	v. s.	660
3-Lauroylamino-2,4,6-triiodo-	15.1	290
4-Acetylamino-3,5-diiodo-	50.0	4,200
4-Benzoylamino-3,5-diiodo-	...	625

It will be noted that acetylation increased the solubility of the sodium salt and decreased the toxicity. Rats were used as the test animals. Toxicity is expressed as LD_{50}, the dosage required to kill 50% of the animals. A high LD_{50} indicates low toxicity. The acyl derivatives of the iodinated

meta-aminobenzoic acid were outstanding in toxicity and solubility; therefore, this series was extended to reveal the member combining the most desirable properties. The data for the 3-acylamino series are more clearly represented by the graphs in Fig. 1.

It is evident that the second member of the series, 3-acetylamino-2,4,6-triiodobenzoic acid, has the lowest toxicity (highest LD_{50}) and its sodium salt has the greatest solubility. Further increasing the size of the acyl group beyond acetyl reduces the solubility of the sodium salt and lowers the LD_{50} (increases the toxicity). Studies by Neuhaus, Christman, and Lewis (17) at the University of Michigan showed that in animals the lower members of the series are eliminated rapidly by the kidney, while the higher members are eliminated more slowly by the kidney and are stored longer in the liver. The second member, 3-acetylamino-2,4,6-triiodobenzoic acid (as sodium salt) was tried clinically by Nesbit and Lapides (18), also at the University of Michigan. Their work established this product as a useful urographic agent which the Mallinckrodt Chemical Works later introduced under the name of Urokon Sodium (sodium acetrizoate) (XIV). Considering its high iodine content (65.8% for the sodium salt) Urokon is certainly one of the least toxic iodine compounds known. Its properties not only have made it useful for urography but have also encouraged radiologists to apply it for a number of other diagnostic purposes.

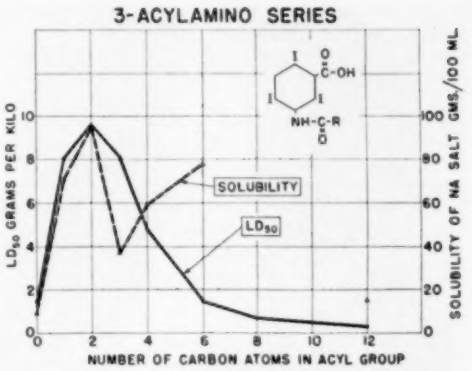


Fig. 1.—Properties of 3-acylamino-2,4,6-triiodobenzoic acids.

MANUFACTURE OF UROKON

It may be of interest to describe briefly the process of manufacturing Urokon. The chemical reactions are exceedingly simple, as shown in the accompanying equations (Eqs. 1–4).

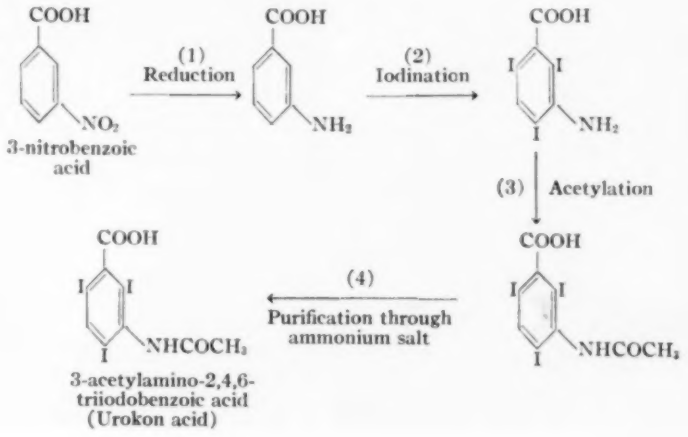
Meta-nitrobenzoic acid is reduced by zinc and hydrochloric acid (Eq. 1). Iodination is accomplished by iodine monochloride (Eq. 2) without isolating the *meta*-aminobenzoic acid. The 3-amino-2,4,6-triiodobenzoic is acetylated (Eq. 3) with acetic anhydride containing a trace of sulfuric acid as a catalyst. Purification (Eq. 4) is accomplished through the ammonium salt using decolorizing carbon. The product, 3-acetylamino-2,4,6-triiodobenzoic acid, is a stable, white, crystalline solid, which melts with decomposition at about 280° and contains 68.5% of iodine.

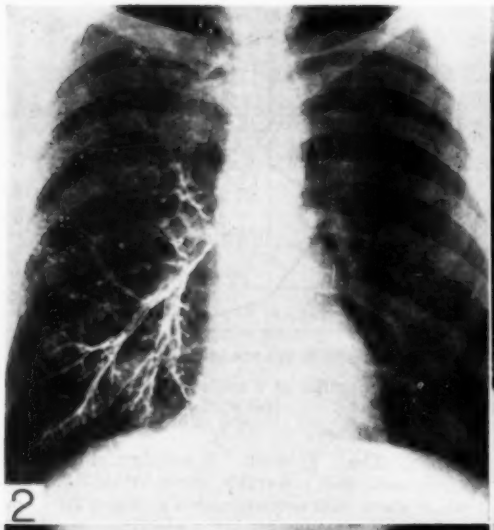
The preparation of Urokon Sodium solution and the filling of ampuls and bottles is carried out in an air-conditioned room lined with glazed tile. The air is purified by a precipitron. The water used in making the solution is tested each day to insure that it is pyrogen free. The ampuls and bottles are sterilized by autoclaving immediately after filling.

APPLICATIONS OF UROKON

The principal use of Urokon is to delineate the urinary system after intravenous injection of a 30% solution. The calices and pelves of the kidneys first become visible (Fig. 4) and later the ureters and the urinary bladder. The use of the more concentrated 70% Urokon Sodium has greatly increased the diagnostic value of intravenous pyelograms. Usually the radiograms obtained with this material are so clear and complete that it is not necessary to subject the patient to the more tedious retrograde examination.

Subcutaneous urography using 70% Urokon has been studied by Melick and Byrne (19) of St. Louis.





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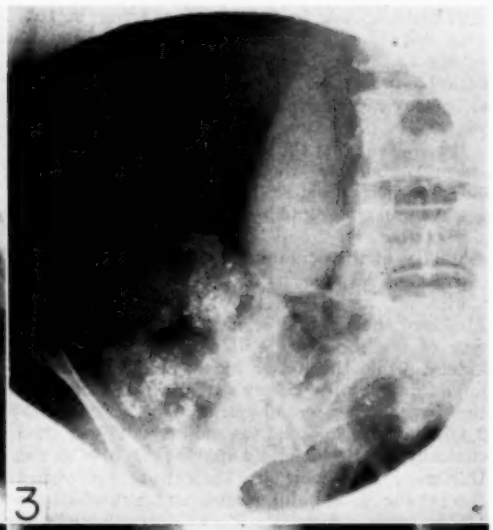

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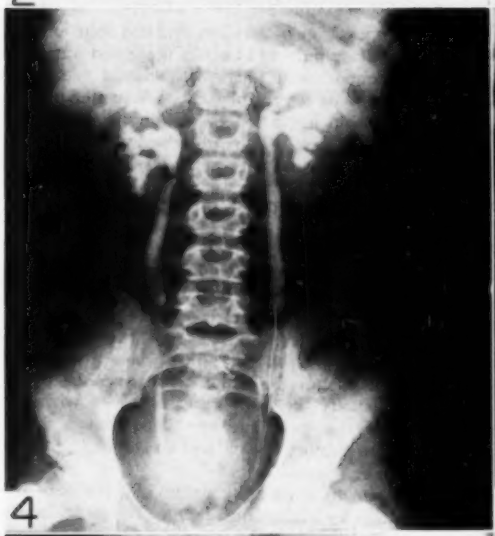

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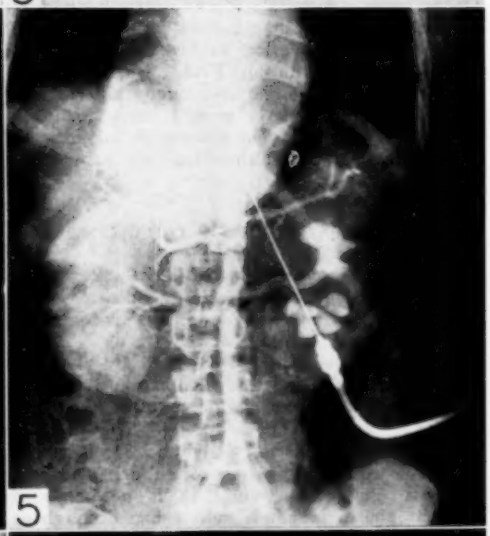

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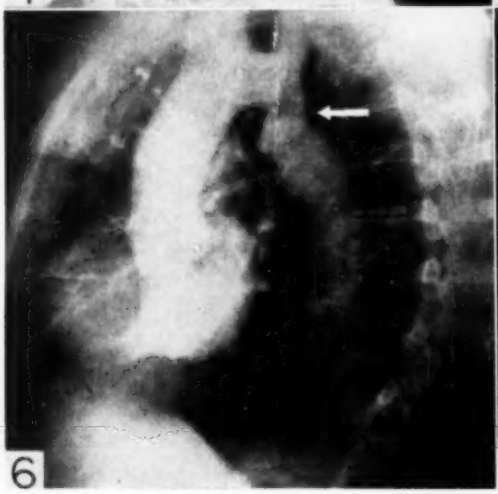

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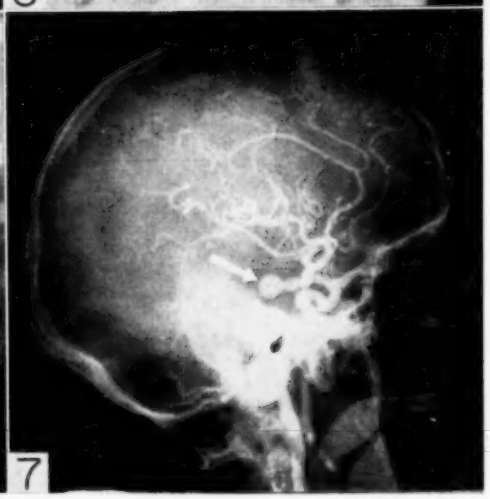

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The procedure has advantages in the case of young children in whom it is sometimes difficult to make an intravenous injection. Simultaneous injection of hyaluronidase accelerates absorption of the contrast medium.

By employing a large dose of the contrast medium in concentrated form and taking the X-ray photographs immediately following injection, the whole body or parenchyma of the kidneys becomes discernible. This branch of radiography, called nephrography, has been the particular interest of Wall and Rose (20) of the Washington University School of Medicine and of Bohne and Christeson (21) at the Henry Ford Hospital in Detroit. The cycle of events comprises three phases: the arterial phase, during which the contrast medium fills the aorta and associated vessels; the capillary phase, when the filling of the small blood vessels opacifies the whole kidney, and the excretory phase, in which the collecting system is filled to give a pyelogram. Following a nephrogram, the pyelogram obtained is of superb quality. It was in such studies as these that the advantages of larger doses of Urokon in intravenous pyelography were first observed. In these investigations the gall bladder was also frequently delineated.

Urokon Sodium 70% has been found particularly suitable for translumbar arteriography (Fig. 5). In this procedure the contrast medium is injected directly into the aorta and delineates the arterial system supplying blood to the kidneys. Melick, Byrne, and Boler (22), Doss (23), and Berry, White, and Metcalfe (24), have recently published contributions to this branch of radiography. The resulting arteriogram often reveals information obtainable in no other way. Renal arteriography has also been accomplished by injecting the medium into the aorta by means of a catheter inserted through the femoral artery as recently reported by Hooke and Graves (25) of Galveston, Tex.

In a recently published study, Rigler (26) and his associates at the University of Minnesota Medical School have demonstrated a new technique for visualizing or opacifying the liver and spleen. Urokon Sodium 70% is injected into the aorta at the level of the eleventh vertebra, somewhat higher than in translumbar arteriography. By sequential roentgenograms, he was able to demonstrate the opacification of the liver and spleen and to correlate the roentgenographic findings with pathologic conditions, such as cirrhosis of the liver, carcinomatous metastases, and kidney dysfunction.

Porporis (27) and the group at Washington University have recently reported the opacification of the liver and spleen by injection of Urokon Sodium 70% intravenously instead of directly into the aorta. Their films demonstrated satisfactorily the abdominal aorta and its visceral branches, the opacification of the liver, spleen, and gall bladder, as well as excellent urograms and cystograms. With regard to the biliary tract, they state that the opacities compare favorably with those obtained in patients who have been examined with the usual oral cholecystographic media.

One of the most spectacular diagnostic procedures is angiocardiography, the radiography of the heart, itself, and associated large blood vessels. Recent studies have been reported from the Washington University School of Medicine under the direction of Scott (28) and by Dotter (29) at the Cornell Medical Center, New York. Investigators at both institutions report fewer side reactions from Urokon Sodium 70% than from other media. The contrast medium, usually 50 cc., is injected rapidly into a large vein, for example, the antecubital vein, and a number of X-ray pictures are taken in rapid succession. Several patients have received as much as 150 cc. of 70% Urokon within the period of one hour without untoward effects. Figure 6 is a typical angiocardiogram showing a constriction (coarctation) of the aorta. Angiocardiography is useful in revealing malformations of the heart and associated large blood vessels, notably those amenable to surgical correction.

Urokon has been used successfully in cerebral arteriography although this procedure has not yet been widely employed. Recent publications are by Gass and Jacobson (30) of Detroit and by Seaman and Schwartz (31) of St. Louis. The cerebral arteriogram shown in Fig. 7 reveals an hour-glass aneurysm, an arterial dilatation caused by the pressure of the blood on weakened tissue.

The literature records other important applications made possible by the low toxicity of Urokon and its relative freedom from side effects even at high dose levels. I will only mention the important advances being made by Boyce, DeTar, and Vest (32) at the University of Virginia and by Abeshouse and Ruben (33) of Sinai Hospital in Baltimore, through their exploration of the veins of the pelvis and the lower extremities.

The procedures involving the injection of a contrast medium into an artery or vein require precise timing and often the taking of several X-ray pictures in rapid succession. For aortography an interesting apparatus for determining the exact moment when an injected contrast medium has arrived at a certain point in the blood circulatory system has recently been developed at Washington University (27). The apparatus consists of a scintillation counter, coupled with an amplifier and a graphic recorder. Diiodofluorescein, tagged with radioactive iodine, is injected together with the Urokon. The scintillation counter is placed over the femoral artery. A swing of the recorder needle indicates the proper moment for the first X-ray exposure. Tagged Urokon has also been used in place of the diiodofluorescein.

Fig. 2.—Bronchogram made with Lipiodol. Fig. 3.—Cholecystogram made with Priodax. Fig. 4.—Intravenous pyelogram using 70% Urokon. Fig. 5.—Translumbar arteriogram by use of 70% Urokon showing poor blood circulation to left kidney which contains staghorn calculi. Fig. 6.—Angiocardiogram made with 70% Urokon. Arrow points to a constriction in the aorta. Fig. 7.—Cerebral arteriogram made with 30% Urokon showing hour-glass aneurysm or dilation of blood vessel. Figs. 2, 3, 4, and 7.—Courtesy of the Washington University School of Medicine. Fig. 5.—Courtesy of Dr. W. F. Melick. Fig. 6.—Courtesy of Dr. Charles T. Dotter.

IN CONCLUSION

I have been able to present only a portion of the thirty or more investigations on Urokon that have so far been published. Examination of the results obtained may indicate limitations of the known contrast media and suggest requisites yet to be fulfilled. In the future we may expect improved media and new techniques to expand the usefulness of roentgenography. The human body, however, possesses only a few mechanisms or physiological processes capable of transporting a contrast agent and concentrating it sufficiently to render an organ opaque. For the immediate future the greatest advances may well be in arteriography and venography, aided by serial roentgenography and a means for precisely timing the pictures. Presently available media may adequately serve this purpose.

It will perhaps be of interest to those engaged in research or the direction of research to reflect on the circumstances leading to the discovery of Urokon. It is a comparatively simple compound in structure. Chemically speaking, it has remained just around the corner from the main pathway of chemical development. Its discovery may best be ascribed to "serendipity," a term coined by Horace Walpole. According to legend, three princes of Ceylon or "Serendip," as it was called, were continually making valuable deductions and discoveries in their travels. It should be added that their discoveries were not accidental or merely by luck, because the king had wisely provided that the princes were carefully trained by selected tutors. In the case of Urokon, the chances of discovering a new and useful compound might have seemed remote, in view of the toxicity of benzoic acid and the extensive explorations we and others had made among closely related compounds. I doubt if the investigation leading to Urokon would have been undertaken, if the chances of success had been carefully weighed. An incipient idea is a delicate thing, likely to shrivel in the blast of budgetary control, market analyses, or even literature surveys. Most research workers have the essential urge to create something. If permitted occasionally to test their own ideas, without first having to defend or justify them to others, they work in a paradise. Denied this privilege, they tend to become frustrated and unproductive. Cooperative research and proper evaluation of projects are important and of course we should not do without them, but my plea is for that small degree of freedom which so encourages the individual research worker to do his creative best.

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Studies in Ergot. I. Ergotamine Phthalate*

By GORDON H. SVOBODA and GEORGE S. SHAHOVSKOY

A method is described for the isolation of ergotamine and its purification as the very stable phthalate salt. The phthalate of ergotamine is formed in preference to most of the other ergot alkaloids, and allows for its ready purification from even the most crude mixtures. The critical physical constants for this salt are described.

TO DATE THERE has been only one method available for the isolation of ergotamine from rye ergot (1), and this has been challenged in the past (2). It has been the purpose of this investigation to develop a readily reproducible method for the isolation and purification of ergotamine.

EXPERIMENTAL

In general, the procedure used consisted of defatting ground cultured Central European ergot with benzene in the presence of aluminum sulfate. The defatted drug was then made alkaline and extracted with benzene. The alkaloids were precipitated in a crude amorphous form from the concentrated percolate by the addition of Skellysolve B. In order to overcome the difficulties inherent in the isolation and purification of ergotamine as the tartrate and similar salts of aliphatic dicarboxylic acids, ergotamine was isolated as the very stable phthalate salt. The phthalate could be isolated from the most impure mixtures, and was never accompanied by more than traces of the physiologically inactive ergotaminine.

The precipitate was extracted with ether and was converted to the crude amorphous phthalate by the addition of an ether solution of phthalic acid.

Mixing of the dried phthalate with acetone resulted in an almost immediate crystallization. The crude crystalline phthalate was recrystallized from acetone as transparent blades with parallel extinction, positive elongation, and a profile angle of 82°, $n_1 = 1.630$, $n_3 = 1.545$, m. p. 182° (dec.), $[\alpha]_D^{25}$ +62° (1% in CH_3OH).

X-ray diffraction data on this compound were obtained by the use of a Norelco 114.59-mm. diameter camera and vanadium oxide filtered chromium radiation ($\lambda = 2.2850$ *A*). The sample was mounted in a parlodion capillary with an inside diameter of 0.4 mm.

Ergotamine phthalate was readily recrystallizable from acetone, methyl ethyl ketone, and acetonitrile.

Anal. Calcd. for $C_{41}H_{41}N_5O_9 \cdot 2H_2O$: C, 62.78; H, 5.74; N, 8.93. Found: C, 62.63; H, 5.61; N, 9.03.

TABLE I.—X-RAY DIFFRACTION DATA FOR ERGOTAMINE PHTHALATE

Interplanar Spacing, *d*	Relative Intensity, I/I_1	Interplanar Spacing, *d*	Relative Intensity, I/I_1
13.3	1.00	4.39	0.40
12.1	0.40	4.57	0.10
10.6	0.40	4.40	0.10
9.32	0.40	4.15	0.10
8.82	0.40	3.90	0.20
8.31	0.10	3.68	0.60
7.30	0.60	3.50	0.10
6.23	0.20	3.30	0.10
5.30	1.00	3.10	0.10

Electrometric titration of the phthalate in 66% dimethylformamide solution showed three groups with the following $pK\alpha'$ values: 4.3, 6.2, and 8.9.

Ergotamine phthalate is evidently composed of one molecule of base and one molecule of acid. Its apparent molecular weight is 780 ± 8.

The infrared absorption spectrum of a sample of ergotamine phthalate is included below.

The free base was readily recrystallized from benzene as transparent *o*-rhombic prisms, $\alpha = 1.636$, $\beta = 1.616$, $2V = 55$–$60°$. The base crystallized with 18.5–19% of benzene of crystallization, and had a specific rotation of $[\alpha]_D^{25}$ −143° (1% in $CHCl_3$). The solvent-free base had a specific rotation of $[\alpha]_D^{25}$ −166°.

X-ray data for a typical free base crystal are given in Table II.

TABLE II.—X-RAY DIFFRACTION DATA FOR ERGOTAMINE BASE

d	I/I_1	*d*	I/I_1
10.7	0.80	4.61	0.30
9.15	0.30	4.56	0.30
7.95	1.00	4.34	0.20
7.63	0.40	4.11	0.80
7.22	0.40	3.99	0.20
6.88	0.30	3.85	0.10
6.46	0.40	3.73	0.20
6.19	0.20	3.61	0.10
5.93	0.30	3.52	0.10
5.49	0.50	3.45	0.10
5.30	0.50	3.40	0.10
5.08	0.20	3.30	0.10
4.95	0.20	3.23	0.20
4.77	0.40	3.09	0.10

PROCEDURE

Two kilograms of moderately coarsely ground cultivated Swiss ergot were intimately mixed with a solution of 200 Gm. of $Al_2(SO_4)_3 \cdot 18H_2O$ in 300 cc. of water. This moist mixture was then percolated with benzene to remove the fat and other extraneous material, approximately 24% of the original weight of drug having been collected as a dark yellow fatty residue. A slow stream of anhydrous ammonia was passed through the drug covered with

* Received December 13, 1952, from the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

The authors are grateful to Drs. S. F. Kern and H. A. Rose for the crystallographic studies; Dr. H. E. Boaz for the infrared absorption spectrum; Mr. D. O. Woolf, Jr. for electrometric titrations, and Messrs. H. L. Hunter W. L. Brown, G. M. Maciak, and J. M. Goodyear for the analytical data.

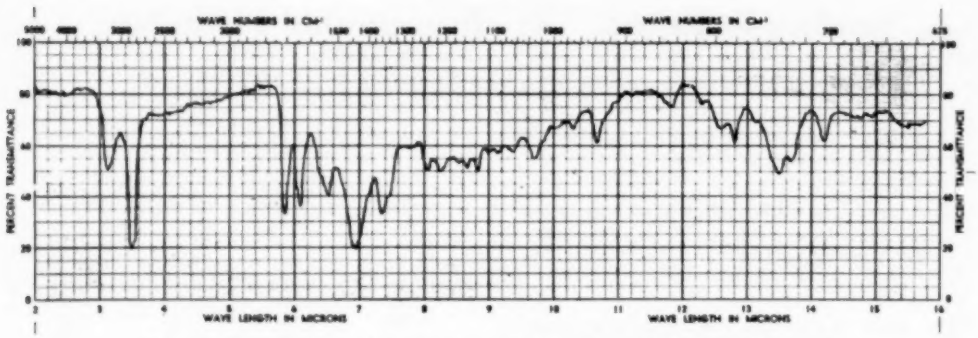

Fig. 1.—Infrared absorption spectrum of ergotamine phthalate—Nujol mull.

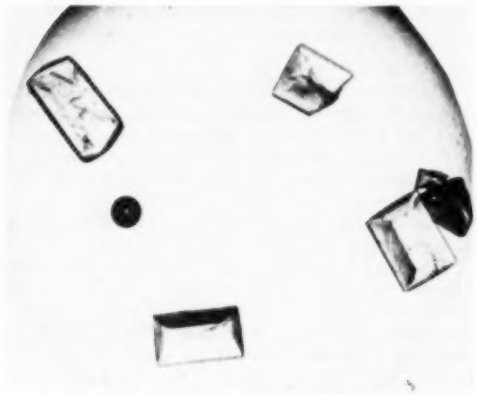

Fig. 2.—Typical ergotamine crystals.

benzene until the drug itself was weakly alkaline. The drug was then percolated to practical exhaustion with benzene—The percolate was concentrated *in vacuo* to a volume of 75–100 cc. Chilling overnight did not induce crystallization. The addition of 1 L. of Skellysolve B precipitated 6.3 Gm. of a light tan amorphous powder.

This crude base was then dissolved in 1,000 cc. of ether and the crude amorphous phthalate was precipitated by the addition of a solution of 2 Gm. of phthalic acid dissolved in 300 cc. of ether containing 10 cc. of methanol. After being chilled, the phthalate was filtered and dried, and 3.7 Gm. of a light tan amorphous phthalate was obtained. This dry powder readily crystallized upon the addition of acetone. Recrystallization of the crude crystalline material from acetone yielded 2 Gm. of transparent rhombic crystals which had the above-cited properties.

SUMMARY

The isolation of ergotamine as the very stable phthalate salt is described. This salt is readily recrystallizable from acetone without decomposition. Ergotamine can be isolated preferentially to most of the other ergot alkaloids as this new phthalate salt.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation of the Illinois Institute of Technology, 35 West 33rd Street, Chicago 16, Ill., seeks information on sources of supply for the following chemicals:

Gymnemic acid
Chavibetol
Melibiase
Invertase
Glycinnic acid
Laccase
Thyroxin
Corypha
Ethaverine
2-Keto-L-gulonic acid
Iron abietate
Chromium-2-ethylhexoate
Pyrazine
2,5-Furandicarboxylic acid
9,10-Diaminoanthracene
4-Hydroxy-1,9-anthrapyrimidine
2-Hydroxycaprylic acid
para-Chlorophenylhydrazine hydrochloride
Acetol
Propionaldol

A Study of Suppository Bases Using Radio-Iodine to Compare the Rate of Absorption from Rectal Suppositories Administered to Rats*,†

By C. F. PETERSON,‡ C. O. LEE, and J. E. CHRISTIAN

A method has been described which determines the rectal absorption rate of a small dose of iodide, using radio-iodine. The radioactive tracer technique made possible a number of determinations for statistical treatment of the data, analyzing the significance of the different amounts absorbed from each base tested.

ONLY A FEW studies have been made which compared the rates of absorption from various suppository bases. The use of cocoa butter was recorded as early as 1752 (1) and is still the principal base used. In recent years substitutes have been proposed, mainly in Germany, as a result of the wartime restrictions of trade and the desire for "ersatz" products. It was soon observed, however, that the new bases altered the rate of absorption.

Following are reports of studies which may be regarded as contributions to the development of the idea that the base influences rectal absorption. Rapp (2) reported in 1927 that aqueous soluble materials were rapidly absorbed from a cocoa butter base containing emulsified water. Schroff (3) and Oesch (1) confirmed that sodium salicylate, sodium iodide, and even salicylic acid were rapidly absorbed by emulsifying the aqueous solution of the drug with lecithin or cholesterol in cocoa butter.

Postonal (a high molecular weight polyethylene oxide) was introduced in Germany in 1935 by Middendorf (4) as a water-soluble suppository base. Freudweiler (5) reported tests by Middendorf that demonstrated a much higher rate of absorption of methylene blue from a Postonal suppository than from cocoa butter.

Charonnat, *et al.* (6), found that methyl nicotinate was absorbed rectally most readily from a glycero-gelatin base, slightly less readily from a cocoa butter base, and incompletely with erratic results from a polyethylene oxide base. The level of the drug in the guinea pigs was noted by the temperature rise measured by a thermocouple placed on the ear.

Experiments by Friesen (7) compared the time for the relief of gallstone pain after the administration of belladonna extract, morphine hydrochloride, and Pantopon separately compounded in suppositories of the following bases: (*a*) cocoa butter by fusion, (*b*) cocoa butter by compression process, (*c*) Postonal 16, glycerin and lanolin 5 parts each, by fusion, and (*d*) Postonal with 10% water by fusion.

The suppositories made by compression provided the most rapid response of the patients to the action of the drugs, followed by those made by fusion of cocoa butter. The Postonal suppositories required a longer time for action of the drug. Base *d* released the drug more slowly than base *c* in spite of the theory, according to Friesen, that fatty substances slow the rectal absorption of drugs.

Hofmann and Hornbogen (8), using normal persons, compared the rectal absorption of 0.5 Gm. sodium salicylate compounded in cocoa butter and four synthetic bases—cetyl phthalate, synthetic paraffin waxes, and two polyethylene oxide bases. These bases permitted greater absorption than cocoa butter. Each base demonstrated a characteristic absorption carve as measured by the urinary excretion. The water-soluble bases yielded a peak level of sodium salicylate in the urine at eight hours and the fatty types at four hours.

EXPERIMENTAL

Five bases were selected for comparison of the rectal absorption of iodide in rats (Table I). The use of the radioactive tracer technique made possible a number of determinations for statistical treatment of the data. The suppository was administered to the rat and after the appropriate time period the rat was killed, and a sample of blood taken, the thyroid removed, and the unabsorbed portion of the suppository collected. The amount of iodide in the blood was used to calculate the total amount of iodide in the extracellular fluid and together with that found in the thyroid was used to express the total iodide present in the animal at that time period. The difference between the amount present in the body (thyroid and fluid) and the amount *not* remaining in the suppository was presumed to have been excreted. It was attempted

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Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† The isotopes used in this work were obtained on allocation from the Atomic Energy Commission.
‡ Smith, Kline, and French Fellow 1950, Purdue University, Lafayette, Ind. Present position: Assistant Professor of Pharmacy, University of Kansas, Lawrence, Kans.

TABLE I.—FORMULAS OF SUPPOSITORY BASES

Base	Source
Cocoa butter	U. S. Pharmacopeia
Glycero-gelatin	U. S. Pharmacopeia, plus 50% water
Carbowax 4000[a]	Polyethylene oxide 4000[a] plus 10% water (Freudweiler, 5)
Propylene glycol monostearate	Propylene glycol monostearate[b] plus 10% triethanolamine stearate (Bird, 9)
Tween 61[c]	Polyoxyethylene sorbitan monostearate[c] plus 10% glyceryl monolaurate[d] (Ward, 10)

[a] Carbowax 4000, Carbide and Carbon Chemical Corporation.
[b] Propylene glycol monostearate, Aldo 25, Glyco Products Company.
[c] Tween 61, Atlas Powder Company.
[d] Glyceryl monolaurate, S 547, Glyco Products Company.

to measure the amount excreted in the urine but it was found impossible to collect the rat urine uniformly.

The radioactive suppositories were prepared by incorporation of 3 μg. of sodium iodide labeled with I^{131}, 1,250,000 counts per minute, per suppository. (The radioactive iodine was obtained from the Atomic Energy Commission after which it was converted entirely to the iodide form.) The melted suppository base was mixed with the sodium iodide and poured into a special mold for twelve 0.3-Gm. suppositories.

Administration of the Suppository.—The rat was weighed and the hair clipped from the area immediately surrounding the anus. The rat was then placed in a holder designed to expose the tail and anus and to keep the rat firmly but gently in place. The rectum was emptied of fecal matter by digital manipulation. The suppository was placed about 2 cm. within the anal opening with the aid of an inserter (Fig. 1). The suppository was held in place by digital pressure until retained by a circular section of rubber tubing slipped over the tail and anus (Fig. 2). The rat was kept in the holder for the allotted time period (one-half, one, or two hours).

Animal Treatment.—The rats used in these tests were fed an iodine deficient diet from weaning time for a period of six weeks. The ration, consisting of ground whole corn, soybean meal, and a vitamin and mineral supplement, was found to contain 43 μg. of iodine per Kg. of ration.[1] The minimal level of iodine is about 200 μg. of iodine per Kg. of ration.

Fig. 1.—Placing of the suppository into the anal opening with the aid of an inserter.

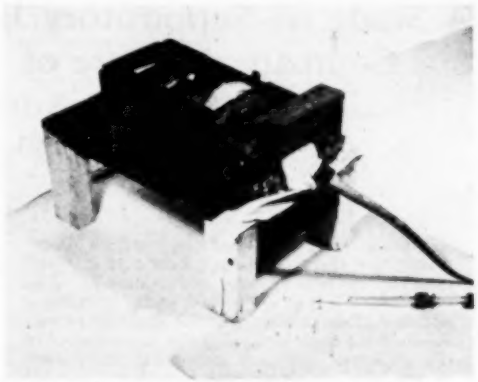

Fig. 2.—The suppository is held in place by a circular section of rubber tubing.

The animals were grouped by sex and age. Rats from each group, selected at random, were administered one of the five suppository bases from the one-half, one, or two-hour period.

Tissue Samples.—The rat was killed at the end of the time period with a blow on the head. The blood (usually 5 cc.) was collected in a tared oxalated beaker and diluted by one-half with 5% sodium hydroxide solution.

The thyroid gland was removed by sectioning the trachea above and below the larynx, insuring the complete removal of the gland. These tissues were washed with water to remove the excess blood, then placed in a 5-cc. vial and filled to the mark with 5% sodium hydroxide solution.

That portion of the suppository which was not absorbed was determined by excising the entire colon distal to the appendix, including the rectum and the skin immediately about the anus. These tissues and contents were placed in a large test tube and 50 cc. sodium hydroxide solution added to extract the iodide.

After a twenty-four hour digestion period, during which the tube was shaken several times, 0.5 cc. of the homogeneous aqueous mixture was transferred to a filter sheet 5 x 6 cm., dried by infrared heat, and sealed under cellophane with scotch tape to a piece of paper only slightly larger than the filter sheet. In the same manner, 0.5 cc. of the diluted laked blood and 0.5 cc. of the digested thyroid gland each were placed on the filter sheets, dried, and sealed.

These radioactive samples were measured using an Eck and Krebs type Geiger-Müller counter, wrapping the sample around the tube. A standard sample was prepared from an appropriate dilution of the radio-iodine in 5% sodium hydroxide solution. The activity of each sample was expressed as the per cent of the amount of radio-iodine administered in the suppository by use of the dilution factors.

[1] By Dr. Herbert Parker, Agricultural Chemistry Department, Purdue University.

TABLE II.—COEFFICIENT OF VARIATION (PER CENT OF THE DOSE DIVIDED BY THE STANDARD DEVIATION)

Suppository Base	Time Period, Hr.	Blood[a] Mean	Blood[a] Coeff.	Thyroid[b] Mean	Thyroid[b] Coeff.	Excreted[c] Mean	Excreted[c] Coeff.	Absorbed[d] Mean	Absorbed[d] Coeff.
Cocoa Butter	½	7.3	0.39	0.98	0.70	2.0	8.0	10.3	0.97
	1	8.4	0.26	1.9	0.90	12.9	0.91	23.2	0.44
	2	8.6	0.30	3.8	0.81	20.4	0.55	32.8	0.50
Tween 61	½	21.3	0.31	2.68	0.90	19.6	0.53	43.6	0.29
	1	33.6	0.33	6.7	0.73	22.4	0.71	62.7	0.22
	2	30.0	0.36	11.1	0.50	28.1	0.70	69.2	0.20
Carbowax 4000	½	26.8	0.31	2.3	0.57	37.3	0.49	66.4	0.29
	1	21.0	0.47	3.6	0.86	39.6	0.23	64.2	0.23
	2	18.9	0.39	8.3	0.75	47.1	0.23	74.3	0.14
Propylene glycol monostearate	½	17.5	0.39	2.2	0.64	29.8	0.24	49.5	0.17
	1	19.1	0.31	4.2	0.62	53.2	0.25	76.5	0.13
	2	23.2	0.28	5.9	0.64	56.4	0.22	85.5	0.08
Glycero-gelatin	½	41.4	0.29	5.2	0.52	27.6	0.41	74.2	0.12
	1	37.5	0.29	9.8	0.30	42.6	0.27	89.9	0.03
	2	33.1	0.27	16.3	0.47	46.0	0.21	95.6	0.02

[a] Blood—Taken from a sample of blood and corrected to represent the per cent of the dose in the extra-cellular fluid (25% of the body weight).
[b] Thyroid—Taken from a sample of the whole thyroid.
[c] Excreted—Calculated from that amount absorbed but not accounted for in the Blood and Thyroid measures (Absorbed minus Blood and Thyroid).
[d] Absorbed—By difference from that remaining in the colon and rectum.

The total amount of iodide absorbed was calculated by subtraction from 100%, the amount found in the remains of the suppository and the associated tissues, the colon, rectum, and anus.

Results.—The bases are compared in Table II by listing the per cent of the dose in each of the three measures and that calculated to have been excreted. With each average, the coefficient of variation is given, which is calculated by dividing the standard deviation by the mean. The coefficient of variation expresses the degree of variation for the different values.

In this table the "Absorbed" measure is the least variable measure and the "Thyroid" measure the most variable. The coefficient of variation remained fairly constant for the "Blood."

The comparison of these five bases was accomplished in two series of experiments, *A* and *B*, on successive months, with every effort to maintain constant technique. Equal numbers of rats, males

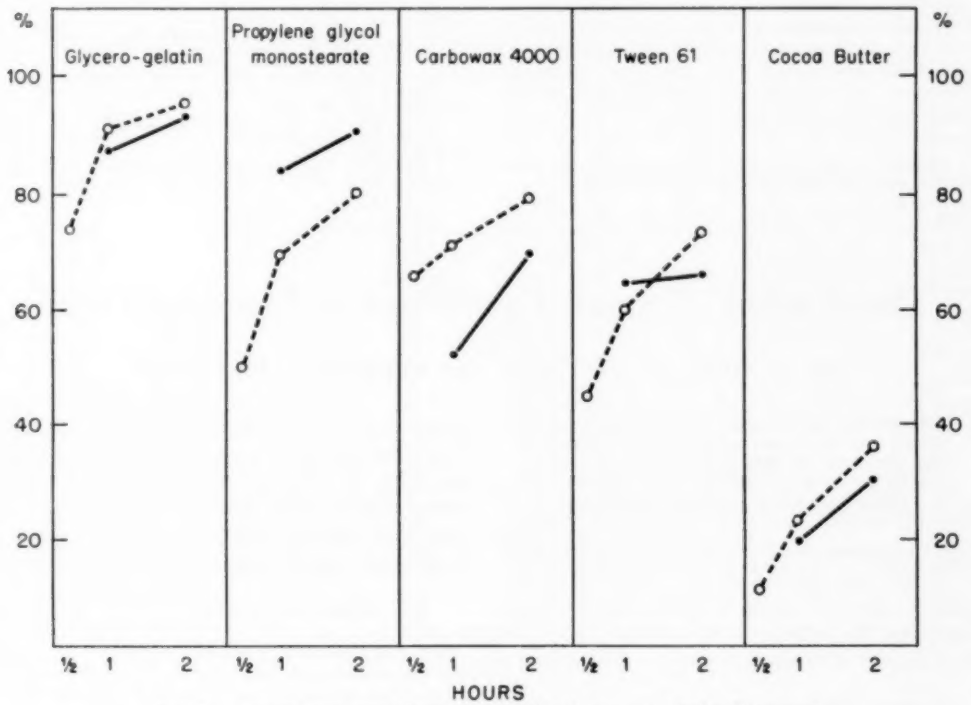

Fig. 3.—Average per cent absorbed. Series *A* ●——● Series *B* ○——○

and females, were used for each of the bases and for both the one and two hour periods in both series. Eight rats per series, per base, per time period were used. In addition, in series *B*, 16 rats per base were killed after one-half hour.

A summary of the absorption from the five bases, showing the average per cent of the iodide dose which was absorbed for each of the time periods in both series is shown in Fig. 3.

Statistical Analysis.—The experiment had been designed to allow for an analysis of variance to determine the significance of the differences.[2] The statistical analysis showed a significant difference at the 1% level for two of the five bases between series *A* and *B* using the method by Snedecor (11). Carbowax 4000 base showed a higher amount absorbed in each measure for the series *B* animals; propylene glycol monostearate base showed less absorbed for the series *B* animals by the "Absorbed" and "Blood" measures, but there was a higher per cent of the iodide in the thyroid for series *B*.

Discussion of Results.—It is very difficult to ascertain the causes of the differences between the two series *A* and *B* for the two bases, since every effort was made to maintain constant technique and to randomize the tests. Carbowax 4000 and propylene glycol monostearate bases were handled with the least difficulty and it is the author's opinion that these bases would be the least likely to show a real difference from one series to another due to changes in manipulation.

A possible explanation is a physiological difference in the groups of animals used for the two series, because there was an increased per cent of the dose in the thyroid for the second series. There was a positive correlation noted between the total absorbed and the per cent of the dose in the thyroid.

Another explanation of the differences between the two series is an inaccurate estimation of the standard dose (which was used to express all measures as the per cent of the dose). An unusually high amount excreted was detected in the first series with the propylene glycol monostearate base, which did not appear with any other base. This observation indicates that it is possible that such an error did occur. Therefore it may be inferred that the per cent of the iodide in the blood and thyroid should have been higher and the per cent of the iodide absorbed lower than those calculated for the first series tests with the propylene glycol monostearate base. These corrections would result in closer agreement of the two series.

[2] The analysis of variance was conducted under the supervision of the Statistical Laboratory, Purdue University.

SUMMARY AND CONCLUSIONS

1. A comparison of the absorption of drugs from various suppository bases has been reviewed to note that the type of base influences the rate of absorption of the drug. From the meager reports in the literature, it appears that each medicament should be tested for the base that would permit its best absorption.

2. A method determining the rectal absorption rate of a small dose of iodide has been described using radio-iodine.

3. The data were analyzed statistically to determine the significance of the different amounts absorbed from each base.

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Distillation of Small Quantities of Essential Oils*

By ROBERT H. REITSEMA and VERNON J. BAARMAN

A kettle and oil separator have been designed which permit rapid separation of essential oils from wet or dry plant materials. From one to ten or more plants can be distilled by use of one of two sizes of apparatus.

AN INVESTIGATION of a large number of essential oils derived from plants of related species and hybrids in the mint family made necessary the isolation of the oil from the plants in a rapid and thorough manner. This required designing new equipment for handling the distillation, since use of flasks as still pots required tedious filling and cleaning operations and most receivers were too wasteful of the oil. Two sizes of equipment were designed in order to take care of single plants and of ten or more plants at a time.

* Received May 7, 1953, from the Chemical Laboratory, A. M. Todd Company, Kalamazoo, Mich.

DESIGN OF EQUIPMENT

The still pots were made from standard pressure cookers of 4 and 20-quart capacities. They were adapted for distillation of mint hay as illustrated in Fig. 1 for the 4-quart, one- or two-plant, size. The

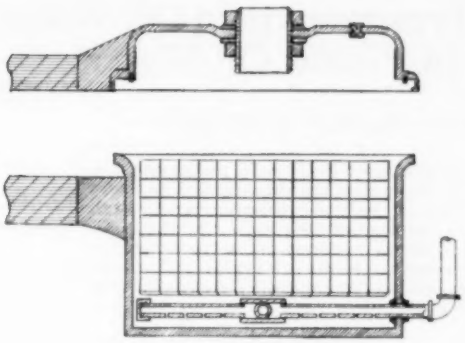

Fig. 1.—Distillation kettle.

center of the top was drilled and a threaded 1.25-inch pipe nipple was inserted and fastened in place with a nut on each side after insertion of an asbestos gasket. The separator was fitted in this pipe with a rubber stopper. The steam was led in through copper tubing to crossed $^1/_4$-inch brass tubing which was drilled with $^1/_{16}$-inch holes at frequent intervals on the bottom side. A wire basket to hold the hay was convenient for removing the spent charge and for keeping the steam from channeling.

The separators were of the type illustrated in Fig. 2. Three points are of importance. The delivery tube from the still pot is bent down at the juncture with the separating part to avoid runback of oil into the still pot. The condensed oil is removed from the water stream during the subsequent distillation as a result of the use of the funnel inside the separator. The oil which rises around the outside of the funnel can be removed by opening the stopcock on the side tube. If only a small amount of oil is obtained, it is desirable to hold all the oil in the separator and remove it through the bottom stopcock at the end of the distillation.

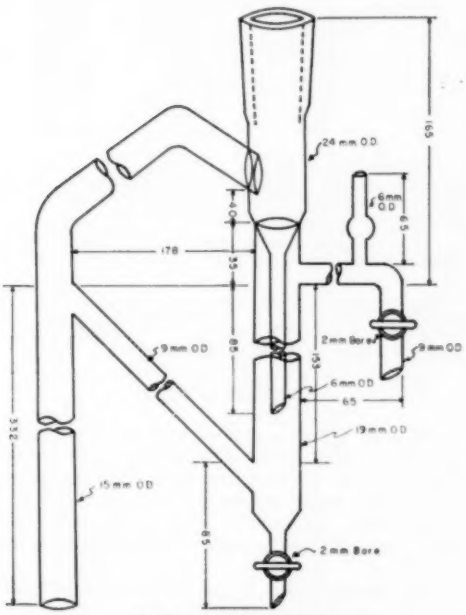

Fig. 2.—Oil separator.

A larger separator has been designed for the 20-quart kettle like the one in Fig. 2, but with larger tubing throughout. One alteration made to permit large throughput was changing the top ground joint from 24/40 to 29/42 and tilting it 30° from the vertical to avoid flooding in the condenser. The condenser in this case must be efficient and we have found the twelve-bulb condensers with one side of the bulbs flattened to be very suitable.

This separator differs from the one described by McKern and Smith-White (1) in raising the oil only to the top of the separator instead of the top of the condenser. One other difference is that the oil is not cooled completely as in their design. This permits the separation of oil to occur at higher temperatures which gives faster and more thorough separation (2). If their apparatus is operated so as to run a warm condensate into the separator there would be losses of volatile material in the space between the condenser bottom and the separator. In the present design the small bore of the air vent on the side tube and the additional distance prevent loss of oil.

Experience has shown that several kettles can be run simultaneously by one man. The kettles are connected to a source of low-pressure steam through brass unions which permit fast removal of the kettles for cleaning. The time of distillation naturally varies with the condition and nature of the hay, but two hours appeared quite adequate for complete removal of the oil.

To test for the odor quality of products a simple design change makes it possible to avoid returning the condensate water to the still pot. In place of the all-glass tubing, a piece of rubber tubing, which can be closed off with a pinch clamp, or a stopcock, was placed in the line running from the bottom of the separator back to the tube going into the kettle. The oil can be removed continuously from the side tube and condensate water removed from the bottom, or oil can be allowed to collect during the distillation. Here, also, the apparatus gives the advantage of use on small amounts of material with maintenance of high recovery, since the oil separated first is not washed with oil-water mixtures later which are poor in oil and could dissolve other oil.

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The Optimum Use of Acid Preservatives in Oil-Water Systems: Benzoic Acid in Peanut Oil-Water*

By EDWARD R. GARRETT and OLIVER R. WOODS

It is shown that the frequent practice of stating mg./cc. of an acid preservative in an oil-water system has *no* bacteriostatic meaning unless the dissociation constant of the acid, the intrinsic distribution constant of the undissociated acid in both phases, the *p*H of the preparation, the relative volumes of the phases, and the minimum concentration of the undissociated acid in the aqueous phase necessary for preservative action are specified. The necessary constants are evaluated for benzoic acid in a peanut oil-water system and conditions for optimum use of this preservative are given.

THE ADDITION of acid preservatives is a standard procedure for the inhibition of yeast growth in the aqueous phase of oil-water emulsions and mixtures. There may be, however, a practical limitation on the amount of acid preservative that may be added per unit of total volume and a frequent problem is the adjustment of conditions to obtain the maximum effective bacteriostasis from this amount. It has been shown by Rahn and Conn (1) that the preservative or bacteriostatic properties of organic acids is a function of the undissociated acid concentration and not of the ion. It follows therefore that a statement of mg./cc. of an acid preservative has *no* meaning as to preservative activity unless are specified (*a*) the dissociation constant of the acid, (*b*) the intrinsic distribution constant of the undissociated acid between the phases, (*c*) the *p*H of the preparation, (*d*) the relative volumes of the phases, and (*e*) the minimum concentration of the undissociated acid in the aqueous phase necessary for preservative action.

The purposes of this investigation were to establish rules for choice of optimum conditions of bacteriostasis in oil-water mixtures and emulsions and to demonstrate their use by the example of benzoic acid in peanut oil and water.

EXPERIMENTAL

Benzoic acid, 2.0418 Gm. (U. S. P.) was dissolved and brought up to 1 L. of volume with water. Varying amounts of 0.1 *N* NaOH and sufficient water to make a total of 5 ml. were added to 20 ml. aliquots of the original solution. Each aliquot was then equilibrated with an equal volume of 25 ml. of peanut oil (Procter and Gamble, crackerjack grade) by thoroughly mixing in a separatory funnel at room temperature. One milliliter of the equilibrated aqueous phase was acidified with 1 ml. of 0.096 *N* HCl and diluted to 100 ml. with water. Ultraviolet spectrophotometric measurements with a recording Cary spectrophotometer at the 229 mμ maximum were made on these last solutions.

In addition, 1 ml. of propylene glycol was added to half of the equilibrated solutions; 3 ml. was added to the other half and the adjusted solutions analyzed similarly to the above.

The *p*H's of all aqueous phases after equilibration were determined with glass-calomel electrodes using a Precision Dow Recordomatic Potentiometer. The experimental data are given in Table I.

TABLE I.—DATA ON BENZOIC ACID DISTRIBUTION IN PEANUT OIL-WATER SYSTEM[a]

Aliquot No.	Ml. of 0.1 *N* NaOH Included in 25 Ml. of Aqueous Phase	*p*H of Aqueous Phase After Equilibration	Absorbance (2) (A_w) of 1/100 Dilution of Aqueous Phase at 229 mμ Max. (Acidified)
1	0.0	3.59	0.308
2	0.5	4.32	0.459
3	1.0	4.67	0.637
4	1.5	4.92	0.828
5	2.0	5.12	1.008
6	2.5	5.50	1.212
7	3.0	6.05	1.410
8[b]	3.5	8.44	1.518
9[c]	5.0	11.21	1.577

[a] The 25 ml. of aqueous phase in all aliquots contained the same amount of benzoic acid before equilibration with the oil phase and included the ml. of 0.1 *N* NaOH given in the second column; 25 ml. of peanut oil was used.
[b] Aqueous phase was slightly yellow after equilibration.
[c] Aqueous phase was brownish in color, possibly due to alkaline degradation of peanut oil or saponification products.

The addition of 2% and 6% propylene glycol by volume had no pronounced effect on benzoic acid concentrations in the aqueous phase.

CALCULATIONS

An acid, added to a two-phase oil-water system distributes itself between the phases in the manner shown in Fig. 1. The fundamental postulates are: immiscible phases, no dimerization of the acid in either phase, no dissociation in the organic phase, and concentrations approximately equal to activities (3, 4).

The undissociated or nonionized acid molecules are distributed between the oil and water phases by the partition law

$$k = [HA]_o/[HA]_w = C_o/[HA]_w \qquad (1)$$

where the ratio of the concentrations of undissociated acid molecules in the oil phase to the water phase is a constant called the intrinsic distribution con-

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V_o = Volume of Oil (O) Phase

V_w = Volume of Water (W) Phase

$$\left\{\begin{array}{l} [HA]_o = C_o \\ \quad\Big\downarrow k \\ \hline \\ [HA]_w \underset{}{\overset{K}{\rightleftharpoons}} [H^+] + [A^-]_w \\ \{[HA]_w + [A^-]_w = C_w\} \end{array}\right.$$

$[HA]_o = C_o$ = molar concentration of undissociated acid in oil phase.

$[HA]_w$ = molar concentration of undissociated acid in water phase.

$[A^-]_w$ = molar concentration of dissociated acid in water phase.

C_w = molar concentration of total acid in water phase.

$q = V_o/V_w$ = volume of ratio phases.

$k = [HA]_o/[HA]_w$, the intrinsic distribution constant of undissociated acid between oil and water phase.

$k' = [HA]_o/([HA]_w + [A^-])$, the experimentally observed distribution constant at a given $[H^+]$.

K = the ionization constant of the acid.

Fig. 1.—Distribution of an acid (HA) between oil and water phases and among molecular and ionic species.

stant k. At a given hydrogen ion concentration $[H^+]$, the experimentally observed distribution constant is:

$$k' = [HA]_o/([HA]_w + [A^-]_w) = C_o/C_w \quad (2)$$

where C_o and C_w are the total acid concentrations in the oil and water phases respectively.

The species distribution in the aqueous phase is a function of the pH or hydrogen ion concentration $[H^+]$ and the ionization constant K of the acid:

$$K = ([H^+] \times [A^-]_w)/[HA]_w \quad (3)$$

If the oil phase did not dissolve any acid, the acid concentration of water phase would be the original concentration, C, before extraction and:

$$C = qC_o + C_w \quad (4)$$

where q is the volume ratio of oil to water phase.

The relations of the above four equations may be combined to give:

$$(K + [H^+])/C_w = [(qk + 1)/C][H^+] + [K/C] \quad (5)$$

If an experimentally observed property such as absorbance (A) is proportional to the acid concentration of the aqueous phase (C_w) then these values may be substituted into equation (5) for the concentration values as the proportionality constants cancel. For the experimental data of this paper the absorbances corresponding to C_w and C (i. e., A_w and A) are proportional where the proportionality constant is the molar absorptivity (ϵ) of acidified benzoic acid. Use of the modified equation (6) does not necessitate foreknowledge of this absorptivity.

$$(K + [H^+])/A_w = [(qk + 1)/A][H^+] + [K/A] \quad (6)$$

This equation is of linear form and a plot of $(K + [H^+])/A_w$ against $[H^+]$ will give a straight line of slope $m = (qk + 1)/A$ and intercept $b = K/A$ so that the intrinsic distribution coefficient $k = (mK - b)/qb$ and the absorbance of the aqueous solution if no acid was extracted by the oil would be $A = K/b$.[1]

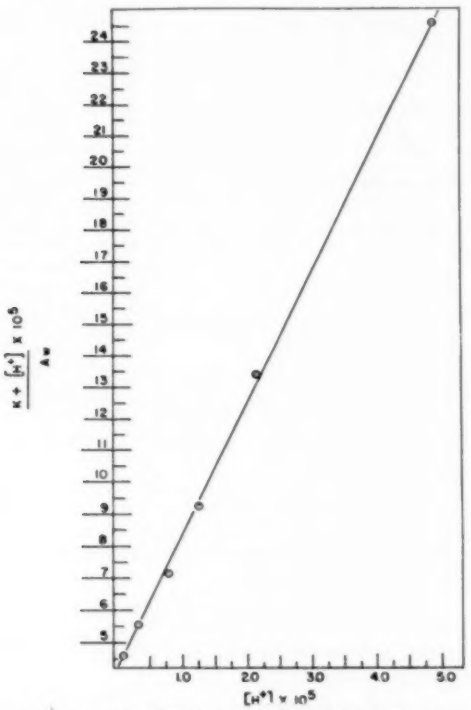

Fig. 2.—Plot of $[(K + [H^+])/A_w] \times 10^5$ against $[H^+] \times 10^5$ for the determination of the intrinsic distribution constant (k) of benzoic acid in the system water-peanut oil where $K = 6.4 \times 10^{-5}$ is the dissociation constant of benzoic acid, $[H^+]$ is the molar hydrogen ion concentration, and A_w is the absorbance of aqueous aliquots at the $[H^+]$.

[1] Such a plot provides more than a method of obtaining a mean value of k from the partition studies over the complete pH scale. Linearity substantiates the premise of no dimerization in the organic phase. For example, benzoic acid does undergo dimerization in many less polar solvents. If K_d is the dissociation constant for benzoic acid dimers in an organic phase: $(HA)_2 \rightleftharpoons 2H.i$, and only monomeric undissociated molecules (HA) can equilibrate between oil and water phases, then equation (5) would be:

$$(K + [H^+])/C_w = \{[qk(1/2 + \sqrt{1/4 + 2(C - C_w)/qK_d}) + 1]/C\}[H^+] + K/C$$

A plot analogous to Fig. 2 would be linear, i. e., of constant slope, only if no significant dimerization of benzoic acid occurs in the organic phase, i. e., $K_d \to \infty$. If dimerization is significant, the slope would be a valid measure of k only when C is approximately equal to C_w, i. e., at high pH values when the greatest portion of benzoic acid is ionized and in the aqueous phase. At lower pH values, C_w becomes significantly less than C; the slope tends to increase and the plot becomes nonlinear.

For benzoic acid $K = 6.4 \times 10^{-5}$ (i. e., pK = 4.2), $[H^+]$ may be calculated from the pH values of Table I in which the absorbances (A_w) are given. The volume ratio $q = 1$ in this instance.

The plot of $(K + [H^+])/A_w$ *vs.* $[H^+]$ is given in Fig. 2, where $m = 4.16$ and $b = 4.22 \times 10^{-5}$ so that $k = 5.33$ and $A = 1.52$. This last figure confirms the experimentally observed absorbance at pH 8.44 where most all benzoic acid should be in the salt form and in the aqueous phase. It may be desired to determine the molar absorptivity ($\epsilon = A/C$) of undissociated benzoic acid. The unextracted aqueous phase was made up as $C = 0.01347$ and diluted 100 times for spectrophotometric measurement so that $\epsilon = 1.132 \times 10^4$ at the 229 mμ maximum.

If the hydrogen ion concentration $[H^+]$ of the aqueous phase is equal to the ionization constant of the acid (K), i. e., pH = pK, then:

$$(a) \quad qk = 2(C/C_w - 1)$$
$$(b) \quad k = 2k' \qquad (7)$$

The pH of the aqueous phase may be adjusted by buffer to the pK of the acid (4.2 in this case) and the two phases equilibrated. The absorbances may be taken before (A) and after (A_w) equilibration or the concentrations determined in both phases (C_o and C_w) after equilibration. Then,

$$(a) \quad k = 2(A/A_w - 1) = 2(1.52/0.42 - 1) = 5.24 \qquad (8)$$

or

$$(b) \quad k = 2k' = 2C_o/C_w = 5.24$$

In case (a) the proportionality constant or absorptivity need not be determined.

If M_o is the number of moles in the organic phase of volume V_o, M_w is the number of moles in the aqueous phase of volume V_w, q is the ratio of volumes (V_o/V_w), the $P\%$ of total moles of acid preservative ($M_w + M_o$) added to the entire two-phase system that is undissociated and in the aqueous phase is given in the expression:

$$100/P = 1 + K/[H^+] + kq \qquad (9)$$

Figure 3 is based on this relation and permits the graphic determination of P at any pH of the aqueous phase expressed as a linear function of pK (i. e., pH = pK $\pm$ n) for any volume ratio (q) of the phases and any intrinsic distribution constant (k). The effectiveness of any acid as an aqueous preservative or bacteriostat depends on this concentration of undissociated molecules in the aqueous phase (1), i. e., $[HA]_w$ which for any given value of kq:

$$[HA]_w = P(M_w + M_o)/100V_w \qquad (10)$$

where all values are known and the P is determined from Fig. 3.

RESULTS AND DISCUSSION

Rahn and Conn (1) have shown that preservative or bacteriostatic properties of acids are functions of the undissociated acid concentration and not of the ion. Thus characterization of a system as to k and K allows choice of the best kind and optimum amount of acid, the best pH and the best volume ratio of the phases for the best preservative action in accordance with the allowable acidity or pH range of the pharmaceutical or food preparation. It is reasonable to believe that the equilibrium of the acid between phases will be similar whether the two phases are separated by one interface or many, i. e., are layered or emulsified.

Figure 3 correlates all factors for any such system. In the particular case studied where $k = 5.33$ and pK = 4.2, the dashed line is characteristic of the system for a volume ratio of phases $q = 1$.

A practical limit of acid preservative is 0.1% sodium benzoate weight to volume, i. e., 1 mg./cc. of sodium benzoate or 0.84 mg./cc. of benzoic acid. Thus, for equal volumes of oil and water in the preparation, there is $2 \times P/100 \times [0.84]$ mg. undissociated benzoic acid in 1 cc. of water phase.

For a given pH the mg./cc. of this total benzoic acid, undissociated and in the aqueous phase, is given in Table II.

TABLE II.—THE MG./CC. OF TOTAL BENZOIC ACID IN WATER-PEANUT OIL SYSTEM (0.84 MG./CC.) THAT IS UNDISSOCIATED AND IN THE AQUEOUS PHASE ($kq = 5.33$)

pH	Mg./Cc. Benzoic Acid Undissociated and in Aqueous Phase
2.5	0.264
3.0	0.263
3.5	0.258
4.0	0.242
4.5	0.202
4.75	0.170
5.00	0.134
5.25	0.097
5.50	0.063
5.75	0.037

Rahn and Conn (1) have shown that it takes 0.25 mg./cc. of undissociated benzoic acid to inhibit yeast multiplication. If we accept this value as the minimum concentration of undissociated acid necessary to prevent bacterial growth in the aqueous phase, it is apparent from Table II that for peanut oil-water the pH of the aqueous phase must be adjusted to below 4.0 to achieve this necessary minimum.

Figure 3 also indicates that reduction of volume ratio (q) of oil phase to water phase could bring the concentration of undissociated benzoic acid in the aqueous phase up to the necessary minimum at a higher pH.

If N is the mg. undissociated acid per ml. of aqueous phase necessary to inhibit yeast growth ($N = 0.25$ mg./ml. for benzoic acid) if M is the amount of acid added per ml. of the two-phase system:

$$PM(1 + q)/100 = N \text{ for } kq \qquad (11)$$

For the specific case (0.1% mg./cc. of sodium benzoate) this becomes:

$$P = 29.75/(q + 1) \text{ for } kq = 5.33q \qquad (12)$$

For various values of q, Table III determined from (12) and Fig. 2 gives the maximum possible pH to obtain the minimum necessary N (0.25 mg. undissociated acid/ml.) in aqueous phase to inhibit yeast growth.

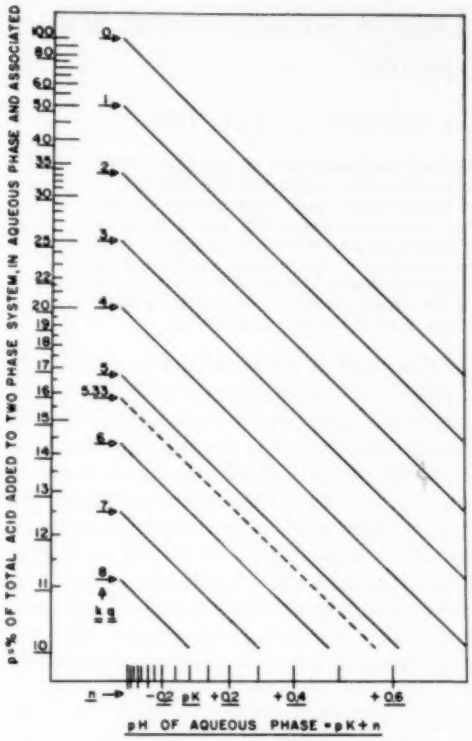

Fig. 3.—Plot of the P % of the total organic acid added to an oil-water system which is in the aqueous phase and undissociated against pH of aqueous phase for given products (kq) of the intrinsic distribution constants (k) and ratios of volumes of oil phase to aqueous phase (q). The pH of the aqueous phase is expressed as a linear relation of the pK of the organic acid, i. e., $p\text{H} = p\text{K} \pm n$.

It is apparent that no matter how little q or how small the volume of the oil phase, the pH cannot exceed 4.56 to obtain the necessary minimum concentration of undissociated benzoic acid in the water phase to inhibit yeast growth.

Figure 3 clearly shows that acids of higher pK and/or lower k values for an oil-water system will show increases in effective concentration of preservative acid in aqueous phase at any given pH.

If pK and k values are relatively constant, then the preservative acid to be chosen must have a lower concentration requirement for the same effective bacterial inhibition.

TABLE III.—MAXIMUM POSSIBLE pH VALUE OF AQUEOUS PHASE TO OBTAIN MINIMUM NECESSARY CONCENTRATION OF UNDISSOCIATED BENZOIC ACID IN AQUEOUS PHASE FOR INHIBITION OF YEAST GROWTH, I. E., 0.25 MG./ML. WHERE $k = 5.33$

Volume Ratio of Oil to Water Phase (q)	pH
1.0	3.7
0.9	4.0
0.8	4.1
0.6	4.26
0.4	4.39
0.2	4.50
0.0	4.56

SUMMARY

1. Numerical and graphical methods are given for the determination of amounts of the bacteriostatically effective undissociated acid in the aqueous phase of an oil-water system. It has been shown that this may be determined for any acid, any pH and any volumes of the two phases by use of only two constants k, the intrinsic distribution constant of the undissociated acid in oil and water phases and K, the dissociation constant of the acid.

2. The methods have been applied to benzoic acid in peanut oil-water in light of practical restrictions on the amount of additive.

3. Spectrophotometric methods of k determination were demonstrated.

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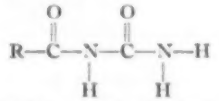
N-Phenyl Ureides of Some Diaryl Amidines of Some Aliphatic Acids*

By JOSEPH G. CANNON† and GEORGE L. WEBSTER

N-phenyl ureides of N,N′ symmetrically disubstituted amidines of aliphatic acids have structures which are isosteric with those of certain sedatives and hypnotics. The amidines themselves are homologs of phenacaine, and their hydrochloride salts may be local anesthetics. Six amidines have been prepared, and a revision of a general method of synthesis, in which less than one molecular equivalent of phosphorus pentachloride is used, has been proposed. An attempt was made to prepare N-phenyl ureides of symmetrical disubstituted amidines in which the N and N′ substituents are different; the failure to obtain these compounds in a pure form is explained on the basis of the tautomerism of the amidine bases.

FEW CLASSES of organic compounds offer such versatility of physiological effects and such a wide spectrum of possible pharmacological applications as do the amidines and their derivatives. In the several years since the preparation of the first synthetic amidines, the compounds, both simple and substituted, have been tested for possible use as local anesthetics, vasoconstrictors, quinidine substitutes, diuretics, and hypoglycemic agents, and as chemotherapeutic agents in the treatment of trypanosomiasis, diphtheria, and tuberculosis. No single compound has been found to possess all of these activities; but, the amidines as a class of compounds have shown decided effects in all of these pharmacological fields.

Of the derivatives of amidines, the N-phenyl ureide derivatives are of interest, because they contain a substituted acyl-like urea nucleus, isosteric with that which is characteristic of certain hypnotics and sedatives.



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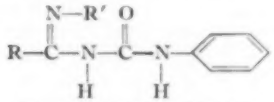

Phenyl Ureide of Amidine

In 1889 Pinner (1) reported the formation of diureide derivatives by the action of phenyl isocyanate on unsubstituted amidines, and the formation of di-thioureides by the action of phenyl isothiocyanate on unsubstituted amidines. Wheeler (2) in 1901 reported the formation of monoureide derivatives of N-monosubstituted amidines, and the preparation of analogous thioureides. Neither Pinner nor Wheeler suggested pharmacological uses for their compounds, and a search of the literature has failed to reveal that compounds of these types were tested pharmacologically, or indeed, whether serious consideration has ever been given to the compounds as physiologically active agents. In 1926 Hill and Rabinowitz (3) suggested that N-phenyl ureides of symmetrical disubstituted amidines of aliphatic acids might have interesting physiological properties; they prepared a number of these phenyl ureides.

It was this possibility of therapeutic usefulness that prompted the present investigation to extend the series of phenyl ureides of Hill and Rabinowitz.

The amidines prepared were homologs of phenacaine, N,N′-bis(*p*-phenetyl)acetamidine, and could be expected to possess some degree of local anesthetic activity. As with phenacaine, the amidine bases were insoluble in water and it was necessary to prepare the soluble hydrochloride salts.

The steps in the synthesis, which is a modification of the method of Hill and Cox (4), are illustrated on the following page.

EXPERIMENTAL

Amides.—A modification of the method of Kuehn and McElvain (5) using one molecular portion of pyridine and the method of Hill and Rabinowitz (3) were used interchangeably. The crude amide was dissolved in ether and the solution was washed with successive portions of ice cold 5% hydrochloric acid, water, 5% sodium hydroxide, and water; this treatment removed much of the color. The ether was then removed from the amide on a steam bath; an alcoholic solution of the residual solid was decolorized by treatment with activated charcoal and subsequent filtration and crystallization from hydroalcoholic solution. See Table I.

Amidine Bases.—The ratio of reactants used was: amide, 0.025 moles; amine, 0.025 moles; phosphorus pentachloride, 0.0216 moles. The phosphorus pentachloride and about 50 ml. of sodium dried benzene were placed in a dry round-bottom flask equipped

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Abstracted from a thesis submitted by J. G. Cannon to the Graduate College of the Chicago Professional Colleges of the University of Illinois, in partial fulfillment of the requirements for the degree of Master of Science.

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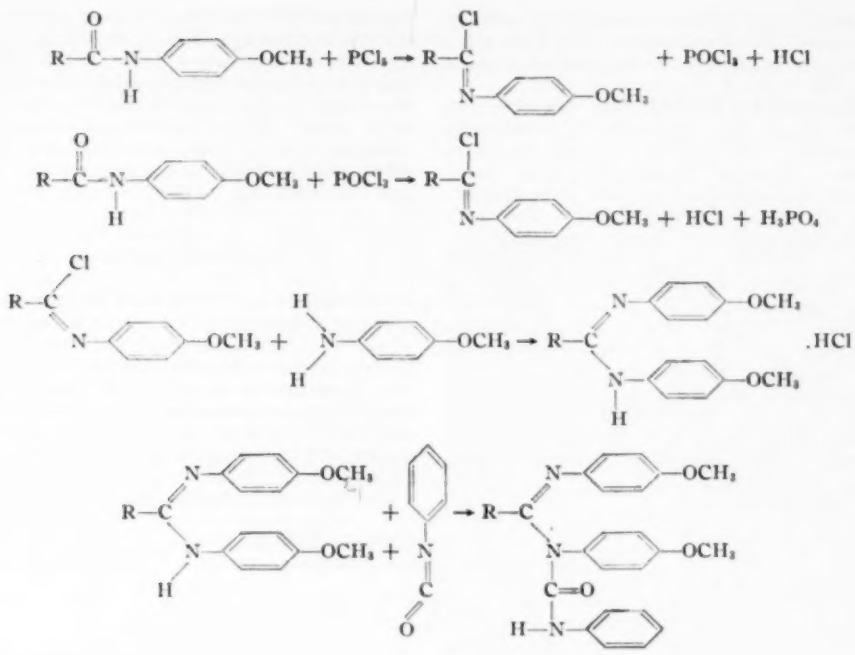
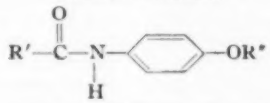


with a condenser fitted with a calcium chloride drying tube, and the mixture was digested on a boiling water bath. When only a negligible amount of hydrogen chloride was being evolved from the mixture (usually after about an hour), the apparatus was removed from the boiling water bath, cooled well, and the amide was added; immediately thereafter, the amine, dissolved in a small amount of sodium dried benzene, was added. Digestion on a boiling water bath was resumed, and hydrogen chloride was soon given off in copious amounts. At the end of the digestion period of three hours, the reaction mixture varied in color from light yellow to brownish orange. In a few cases, the amidine hydrochloride separated from the benzene as a heavy dark amber liquid phase and, after cooling thoroughly, the benzene layer was decanted and discarded. However, in most cases, the amidine hydrochloride seemed to be soluble in benzene.

The benzene and remaining phosphorus oxychloride were removed from the reaction mixture by distillation at reduced pressure from a boiling water bath. The residue was twice mixed with 50-ml. portions of sodium dried benzene and each portion again distilled off. The crude amidine hydrochloride was dissolved in an excess of warm 95% ethanol, and a small portion of this solution was transferred to a test tube. The test tube and its contents were thoroughly cooled in an ice-water mixture, and concentrated ammonium hydroxide solution was added in small portions, to precipitate the amidine base. (These bases, without exception, were thrown out of solution as heavy yellow oils.) With continued cooling and vigorous scratching of the inside of the test tube with a heavy glass rod, the amidine base was induced to crystallize. The small amount of amidine crystals obtained by this procedure was used to induce crystallization in the remainder of the amidine solution by seeding, after the addition of an excess of ammonium hydroxide.

The crude crystalline base was collected on a filter and was purified by treatment of its solution in hot alcohol with activated charcoal, and subsequent recrystallization from aqueous alcohol. All of the

TABLE I.—AMIDES



Amide No.	R′	R″	Yield, %	M. p., ° C., Uncorr.	N Analysis, %[a] Calcd.	N Analysis, %[a] Found
1	*n*-propyl	Methyl	76	86–89	7.25	7.12
2	*n*-butyl	Methyl	65	83–85	6.76	6.77
3	Isopropyl	Ethyl	71	132–134	6.76	6.78
4	Isopropyl	Methyl	68	108–110	7.25	7.37

[a] Nitrogen analysis by a semimicro Kjeldahl method.

bases prepared were soluble in alcohol, ether, acetone, and benzene, and in hydrochloric acid, from which they could be precipitated by the addition of excess alkali. See Table II.

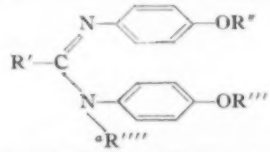
Phenyl Ureides of the Amidines.—The well-dried, purified amidine base was dissolved in a minimum amount of dry ether contained in a glass-stoppered container. An equimolecular portion of phenyl isocyanate was added, and the mixture was permitted to stand at room temperature. After some minutes, the phenyl ureide began to crystallize out, usually as granules. At the end of twenty-four hours, the mixture was placed in a refrigerator to complete crystallization, then the solid was collected on a filter. This crude product was recrystallized from boiling ether. It was necessary to recrystallize two of the phenyl ureides several times in order to obtain products that melted sharply; this greatly lowered the yields. All of the phenyl ureides were sparingly soluble in cold ether, more soluble in boiling ether, and easily soluble in acetone and ligroin. They were insoluble in water and in hydrochloric acid. See Table II.

Hydrochlorides of the Amidines.—Dry hydrogen chloride gas was bubbled through a portion of dry ether for about a minute. This ethereal hydrogen chloride solution was added in small amounts and with agitation to a solution of the dried amidine base in dry ether; in the majority of experiments, the amidine hydrochloride separated as a flocculent solid. Ethereal hydrogen chloride was added until no more precipitate was formed, care being taken not to add more than a slight excess of hydrogen chloride. In those instances when the hydrochloride separated as an oil, crystallization was encouraged by decanting the ether layer, washing the oily hydrochloride with fresh dry ether or ligroin, discarding this, and adding another portion of dry ether or ligroin as the supernatant layer. If this technique was not successful, crystallization was attempted from a saturated alcoholic solution by the addition of dry ether. All hydrochlorides were purified by dissolving them in a minimum amount of hot absolute alcohol, cooling, and adding dry ether in excess. See Table II.

DISCUSSION

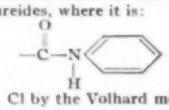
The crude amidine bases could not be crystallized when, as Hill and Cox (4) suggested, slightly more (here, 10% more) than a molecular equivalent of phosphorus pentachloride was used. This excess was presumably for the purpose of compensating for that phosphorus pentachloride decomposed by small amounts of moisture present in the reaction apparatus. It might be surmised that phosphorus pentachloride could function not only to form the imidyl chloride as desired, but might also act to chlorinate other portions of the molecule, thus giving rise to an uncrystallizable mixture of substances. In 1927, von Braun (6) had reported that too great an excess of phosphorus pentachloride led to polychloro substitution on the aliphatic acid portion of the amide. Accordingly, in this investigation, about 10% less than an equivalent amount of phosphorus pentachloride was used in all reactions. This quantity was sufficient to unite with minute amounts of water present in the reaction apparatus, and to produce yields comparable to those which Hill and Cox (4) obtained in the preparation of similar amidines. Phosphorus oxychloride (produced

TABLE II.—AMIDINES AND DERIVATIVES



Amidine	R′	R″	R‴	Yield, %	M. P. Uncorr., °C.	Analyses, %[b] Calcd.	Found
No. 1	*n*-propyl	CH_3	CH_3	83	88–91[c]	N 9.39	N 9.32
—HCl	*n*-propyl	CH_3	CH_3	95	162–164	Cl 10.54	Cl 10.54
—Ureide	*n*-propyl	CH_3	CH_3	quant.	121–124	N 10.06	N 10.31
No. 2	*n*-propyl	CH_3	C_2H_5	64	78–81	N 8.97	N 8.93
—HCl	*n*-propyl	CH_3	C_2H_5	93.5	118–121	Cl 10.16	Cl 10.25
No. 3	*n*-butyl	CH_3	C_2H_5	61.5	72–75	N 8.58	N 8.47
No. 4	*n*-butyl	CH_3	CH_3	62	68–70	N 8.97	N 8.84
—HCl	*n*-butyl	CH_3	CH_3	84	160–162	Cl 10.16	Cl 10.00
—Ureide	*n*-butyl	CH_3	CH_3	30	100–103	N 9.74	N 9.69
No. 5	Isopropyl	CH_3	CH_3	74	66–68	N 9.39	N 9.42
—HCl	Isopropyl	CH_3	CH_3	97.5	184–187	Cl 10.54	Cl 10.56
—Ureide	Isopropyl	CH_3	CH_3	50	127–130	N 10.06	N 10.28
No. 6	Isopropyl	C_2H_5	C_2H_5	70	76–78	N 8.58	N 8.48
—HCl	Isopropyl	C_2H_5	C_2H_5	94	183–185	Cl 9.77	Cl 9.90

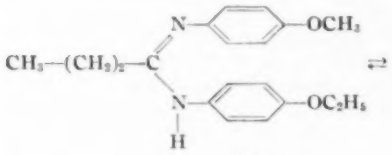
[a] R″″ is H in all compounds except the phenyl ureides, where it is:



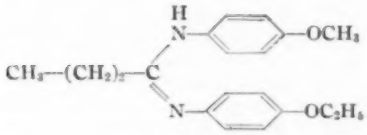
[b] Analyses: N by a semimicro Kjeldahl method; Cl by the Volhard method.
[c] Previously reported by Drozdov and Bekhli (8).

by the reaction of phosphorus pentachloride with water and with amides) also is capable of reacting with the amide to form the intermediate imidyl chloride, and therefore an equimolecular portion of phosphorus pentachloride is not necessary for maximum yields. No attempt was made to determine the minimum amount of phosphorus pentachloride necessary to produce maximum yields. Hontz and Wagner (7) carried out an amidine synthesis using one quarter the equivalent amount of phosphorus pentachloride, in an attempt to make full use of the halogenating powers both of the phosphorus pentachloride, and of the phosphorus oxychloride formed. This reaction produced much tar and low yields of amidine, and was reported as being entirely unsatisfactory.

When an attempt was made to prepare phenyl ureides of those amidines in which the N and N′ substituents were not the same, oily or semisolid products resulted, which could not be crystallized. The probable explanation for this failure to prepare crystalline phenyl ureides of symmetrical disubstituted amidines having unlike N and N′ groups lies in the tautomerism of the amidine bases. Such compounds are composed of two distinct structures in equilibrium with each other:

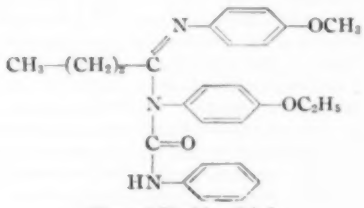


(*a*)

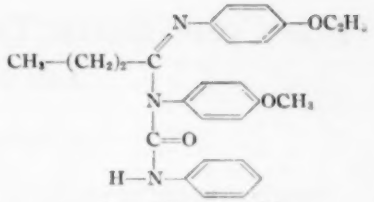


(*b*)

Each tautomer is capable of forming a phenyl ureide on treatment with phenyl isocyanate; the phenyl ureides of the two tautomers are separate and distinct compounds, since the amidine, in linking to phenyl isocyanate, has lost its tautomeric property.



Phenyl Ureide of (*a*)



Phenyl Ureide of (*b*)

It is therefore proposed that the failure to obtain crystalline phenyl ureides of the amidines with unlike N and N′ substituents (No. 2 and No. 3, Table II) was due to the formation of a mixture of phenyl ureides of the tautomers. Such phenyl ureides might well form a "eutectic" mixture, resulting in a semisolid or an oil; and their structures are so similar that it is extremely difficult to separate them by either chemical or physical means. A search of the literature has not revealed reports of attempts prior to this to form phenyl ureides by treating N, N′ dissimilarly substituted amidines with phenyl isocyanate; but it has been shown by Beckmann and Fellrath (9) that the alkylation of a symmetrical disubstituted amidine in which the N and N′ groups were different led to the formation of two products, due to the tautomerism of the starting material. This observation tends to substantiate the proposal that a mixture of phenyl ureides of the two tautomers was formed.

The phenyl ureide of amidine No. 6, Table II, separated as an uncrystallizable oil. No reason could be found for this failure to obtain a crystalline product.

SUMMARY

1. Five new amidine bases have been prepared, and in addition another amidine base previously reported in the literature has been prepared in a new manner.

2. The hydrochlorides of five of these six amidine bases have been prepared for testing as local anesthetics of the phenacaine type.

3. The phenyl ureides of three of the N,N′ similarly disubstituted amidines have been prepared.

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The Colorimetric Determination of Caffeine in Tablet Mixtures*

By ROBERT A. DAOUST†

It has been found that the alkaloid caffeine may be quantitatively precipitated from aqueous solution by phosphomolybdic acid. The caffeine phosphomolybdate may be dissolved in acetone and determined colorimetrically at 440 mμ. The method may be applied to samples containing from 1 to 5 mg. of caffeine and possesses several advantages over the official A. O. A. C. iodometric and bromometric methods. Although not specific for caffeine, the reaction furnishes a useful and rapid method for determining small amounts of the alkaloid in the presence of phenacetin and aspirin in APC tablets. A method for the determination of caffeine in APC tablets with recovery data is described.

A RAPID and simple method for the quantitative determination of caffeine in the presence of phenacetin and aspirin in APC tablet mixtures employing small amounts of samples for assay is a desirable asset for pharmaceutical analysis. The treatment and separation of the three components of APC tablets, and the determination of each, according to the official A. O. A. C. method (1), are time consuming and tedious, and lack specificity to a considerable degree. Phosphomolybdic acid, long known as an alkaloidal precipitant, has not been investigated for the colorimetric determination of alkaloids. Richardson and Campbell (2), and Banes (3) described a method for the gravimetric determination of theobromine in tablet mixtures in which the alkaloidal reagent, phosphotungstic acid, was employed in the quantitative precipitation of insoluble theobromine phosphotungstate. This suggested a similar gravimetric and colorimetric method for caffeine to be investigated with the purpose of developing a rapid, accurate procedure for small amounts of caffeine in APC tablets, but it was found that caffeine phosphotungstate, an insoluble white precipitate, formed a colorless solution when dissolved in acetone. Fortunately, it was noted that another alkaloidal reagent, phosphomolybdic acid (4, 5), formed an insoluble yellow precipitate of caffeine phosphomolybdate (6) which dissolved in acetone giving a yellow colored solution which could be measured on a spectrophotometer. The reaction for this precipitation is probably as follows:

$$24MoO_3.2H_3PO_4 + 4H_2O + 6C_8H_{10}O_2N_4 \rightarrow 2H_7P(Mo_2O_7)_6.(C_8H_{10}O_2N_4)_2$$

EXPERIMENTAL

Reagents.—(*a*) *Phosphomolybdic Acid Solution, 20% (w/v).*—Twenty grams of phosphomolybdic acid (Merck reagent grade) crystals is dissolved in about 50 ml. distilled water, warmed with stirring until a clear yellow solution is obtained, then cooled to room temperature, and diluted to 100 ml. with distilled water. This solution decomposes on exposure to light and should be kept in a well-stoppered, dark amber bottle.

(*b*) *Caffeine Standard Solution.*—One hundred milligrams of anhydrous caffeine alkaloid, U. S. P., previously dried several hours over phosphorous pentoxide in a vacuum desiccator, is dissolved in about 50 ml. distilled water and diluted to the mark in a 100-ml. volumetric flask. Each milliliter of this solution contains 1.0 mg. of caffeine.

(*c*) *Dilute Hydrochloric Acid (1:1).*—Fifty milliliter of concentrated hydrochloric acid is mixed with 50 ml. of distilled water.

(*d*) *Dilute Hydrochloric Acid (1:9).*—Ten milliliter of concentrated hydrochloric acid is mixed with 90 ml. distilled water.

(*e*) *Acetone.*—Reagent grade.

Preparation of the Standard Graph.—Aliquots of 1, 2, 3, 4, and 5 ml. of the standard solution of caffeine are pipetted in order into each of five 50-ml. beakers. One-half milliliter of concentrated hydrochloric acid and sufficient distilled water are added to each beaker to make the volume 11 ml. The beakers are placed on a steam bath and warmed for a few minutes. When hot, 2 ml. of 20% aqueous solution of phosphomolybdic acid is added, drop by drop, from a pipette with stirring of the solution in the beaker. The beakers are covered with watch glasses and heated for fifteen minutes on the steam bath to enable complete precipitation to take place. The precipitate is then filtered with suction through a 15-ml. fritted glass funnel of fine porosity attached to a 250-ml. suction flask. Each precipitate is washed with three successive 5-ml. portions of cold dilute hydrochloric acid (1:9). The filtrate from the last washing should be colorless. All the acid remaining on the precipitate is removed by thorough aspiration.

The precipitates are dissolved in acetone by adding three 5-ml. portions of acetone through the funnel. Each portion if transferred to a 25-ml. volumetric flask by means of a small funnel with a 6-mm. diameter stem long enough to reach the base

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† The author wishes to thank Dr. Jean M. Dunbar, Technical Director, for permission to publish this paper.

of the neck of the flask. The solution in the volumetric flask is filled just short of volume and this is made up to the mark with acetone. Bandelin (7) suggests the technique of dissolving the precipitate in acetone by application of gentle air pressure directly to the surface of the acetone in a 15-ml. fritted glass crucible and collecting the filtrate in a volumetric flask. Another technique (8) using an apparatus with a suction bell jar and volumetric flask may also be satisfactory. The optical density or per cent transmittance of the caffeine phosphomolybdate in acetone may be measured directly at 440 mμ on a Coleman Spectrophotometer, Model 14. The calibration curve of caffeine, plotted on a linear scale at 1-mg. increments in the range from 1–5 mg., indicated that the method obeys Beer's law with good agreement. The absorption spectra of caffeine phosphomolybdate in acetone were found to develop a sigmoid curve when plotted on linear scale.

The advantages of the phosphomolybdate method indicate that it is quite sensitive and as reliable as the official method. The overlapping of the admixed components in the final weighings of the residues by the gravimetric procedure in the official A. O. A. C. (1), and Holt's (9) "shake out and weigh" methods; and the larger samples required in the iodometric (10), and bromometric (6) procedures leave much to be desired in specificity, time, and material. Wirth (10) recommends that a minimum of 240 mg. of caffeine alkaloid in the presence of phenacetin and aspirin be used for a determination by the iodometric titration method.

A concentration of 3 mg. caffeine for each assay was selected as the most suitable, although any amount between 1 and 5 mg. caffeine should serve as satisfactorily for the phosphomolybdate method, but it is preferable to adjust the amount to correspond within the range of optical density between 0.2 and 0.5, or with the range of per cent transmittance between 63 and 31.

Procedure for APC Tablets.—The average tablet weight is calculated from the weight of 20 tablets weighed accurately. Transfer a sample of the pulverized tablets, containing approximately 30 mg. caffeine, into a 250-ml. beaker, and add 100 ml. distilled water. Heat just to boiling with stirring over a burner to dissolve all the sample. Transfer the beaker onto a steam bath, add with stirring 10 ml. of dilute hydrochloric acid (1:1), then add, drop by drop, from a pipette 2 ml. of the phosphomolybdic acid reagent. Cover the beaker with a watch glass and continue heating until the heavy yellow precipitate subsides and the supernatant liquid turns green and somewhat clearer. Complete precipitation should take place within fifteen to twenty minutes. Filter hot with suction through a 30-ml. fritted glass funnel of medium porosity. The yellow precipitate is washed three times with 10-ml. portions of cold dilute hydrochloric acid (1:9). Scrubbing the walls of the beaker with a rubber policeman will aid in transferring remaining particles of the precipitate onto the filter.

The precipitate is dissolved in acetone, added in small portions, stirring the precipitate with a glass rod drawn to a point at one end. The yellow colored filtrate is transferred quantitatively into a 100-ml. volumetric flask and diluted to the mark with acetone. A 10-ml. aliquot is pipetted into a 25-ml. volumetric flask and diluted to the mark with the same solvent. Another technique (8) involving the application of a bell jar and volumetric flask as previously mentioned may be used satisfactorily. The absorbance (A) or per cent transmittance may be measured at 440 mμ. The amount of caffeine present may be determined by reference to the standard graph, or if one prefers, by measuring the absorbance of a standard solution simultaneously with that of the sample.

Calculation:

$$\frac{A \text{ sample}}{A \text{ standard}} \times \frac{3 \text{ mg.}}{25 \text{ ml.}} \times \frac{100 \text{ ml.}}{\text{wt. sample}} \times \frac{\text{Av. tablet wt.}}{1 \text{ tablet}} \times \frac{25 \text{ ml.}}{10 \text{ ml.}} = \text{mg./tablet caffeine}$$

or,

$$\frac{\text{mg.}}{1 \text{ tablet}} \times \frac{1 \text{ Gm.}}{1000 \text{ mg.}} \times \frac{15.432 \text{ grains}}{1 \text{ Gm.}} = \text{grain/tablet caffeine}$$

Table I shows the results obtained when the APC tablets were assayed for caffeine using the previously described procedure. A total of 25 Gm. of APC tablet mixture was prepared by weighing the U. S. P. or C. P. ingredients accurately on the analytical balance. The mixture was triturated thoroughly.

TABLE I.—RESULTS OF ASSAYS FOR CAFFEINE ON KNOWN APC TABLET MIXTURES

Ingredients	Calculated, %	Recovery of Caffeine, %
Caffeine alkaloid	6.06	102.68
Aspirin	48.51	98.20
Phenacetin	30.30	98.90
Powdered acacia	2.15	98.01
Powdered sugar	1.93	
Corn starch	10.48	
Calcium stearate	0.57	

Table II shows the results of the assays made on manufactured APC tablets.

TABLE II.—RESULTS OF ASSAYS FOR CAFFEINE ON MANUFACTURED TABLETS[a]

Caffeine Found, Mg.	Recovery, %
33.28	102.72
32.51	100.34
32.04	98.88
33.07	102.07
32.66	100.80
32.27	99.59
32.79	101.20
32.54	100.43
32.51	100.34
32.19	99.35
32.71	100.94
32.19	99.35
32.14	99.19
32.59	100.59
33.23	102.56
31.90	98.46

[a] Calculated 32.4 mg. claim.

DISCUSSION

By the phosphomolybdate method, determinations can be easily and conveniently carried out on 30-mg. aliquots with reasonable accuracy.

It may be possible, however, to carry out determinations satisfactorily on 3-mg. aliquots by the following modification: The sample of powdered APC tablets, containing approximately 30 mg. caffeine, is dissolved in about 75 ml. distilled water heated to boiling, then cooled to room temperature. The material which settles is centrifuged off and the clear supernatant liquid transferred into a 100-ml. volumetric flask. The precipitate should be washed with small portions of cold distilled water and the washings used to dilute the solution to the mark of the flask. A 10-ml. aliquot is taken for assay by the procedure for the preparation of the standard graph.

It is apparent that aspirin, phenacetin, and other ingredients of APC tablets do not interfere with the colorimetric determination of caffeine. If the caffeine is precipitated from a solution of APC tablets with a large excess of phosphomolybdic acid in the proposed method, a greenish deposit forms on the precipitate due to reduction which interferes with the colorimetric determination. Caffeine precipitated by phosphomolybdic acid in boiling sulfuric acid, according to the procedure described by Banes (3), redissolves rapidly forming a molybdenum blue solution. Long heating, for approximately an hour, using hydrochloric acid in place of sulfuric acid produces the same result.

Nitric acid cannot be used as an acid medium in the assay of APC tablets by the phosphomolybdate method, for it produces with phenacetin a yellow reaction product of 3-nitro-4-acetamidophenetole. This reaction is the basis of a new colorimetric method for phenacetin (11).

It is possible that in other pharmaceutical preparations, caffeine may be separated from interfering substances, such as antipyrine (12), and other antipyretic drugs and alkaloids by the chromatographic adsorption technique.

Extraction with organic solvents in the case of APC tablets is eliminated in the phosphomolybdate method. The tablet mixture dissolves readily in the hot acid solution with a slight opalescence which does not interfere with the colorimetric determination.

The standard graph of caffeine shows that amounts ranging from 1–5 mg. of the alkaloid correspond to values of optical density varying from 0.086–0.432 measured at 440 mμ on the Coleman Spectrophotometer. Photoelectric colorimeters provided with blue filters transmitting in the region of 440 mμ should give satisfactory results.

SUMMARY

Caffeine may be determined quantitatively in the presence of aspirin and phenacetin by precipitation from dilute hydrochloric acid solution with phosphomolybdic acid. After filtration the caffeine phosphomolybdate is dissolved in acetone and measured colorimetrically at 440 mμ using a spectrophotometer or suitable photoelectric colorimeter. A standard graph may be prepared for caffeine. A method for APC tablets is given. The phosphomolybdate method is sensitive and gives reliable results on small samples.

Various antipyretic drugs and alkaloids may interfere by forming insoluble phosphomolybdates, which may possibly be separated from caffeine by chromatographic adsorption technique.

The phosphomolybdate method may possibly be applied with success to other alkaloids which do not respond quantitatively to other well-known reactions.

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Extraction of Ergot Alkaloids by Tetrahydrofuran and Its Mixtures*

By J. M. CAMPO† and L. G. GRAMLING‡

The results of a study of the extraction of ergot alkaloids from the crude drug by tetrahydrofuran, tetrahydrofuran-water azeotrope (4.3 per cent water), and tetrahydrofuran-water solution (50 per cent v/v) is presented. The preparation of a tetrahydrofuran-chloroform azeotrope is reported and its composition determined by index of refraction measurements. This azeotrope yielded the highest degree of alkaloidal extraction of any of the tetrahydrofuran mixtures employed. The selectivity of these solvent mixtures for color principles and emulsion-forming constituents of the crude drug is also discussed. All original extractions were carried out in the absence of alkalinity. Thus the ability of the solvents to extract the alkaloids as they occur naturally could be determined.

CARKHUFF (1), in an investigation of various furans as solvents for the extraction of belladonna root, reported the most favorable selectivity for l-hyocyamine by Soxhlet extraction of the ground drug with tetrahydrofuran-water azeotrope. These findings, together with the reported results that tetrahydrofuran in combination with water shows a greater selectivity for caffeine than either solvent alone (2), suggested a further investigation of tetrahydrofuran mixtures for drug extraction.

It was decided to investigate the selectivity of this solvent in the extraction of ergot alkaloids from the crude drug. Whole Spanish ergot, purchased from a reputable distributor, was reduced to a No. 60 fineness and then defatted with petroleum ether. All extractions in this investigation were made from this prepared lot. The tetrahydrofuran which is available commercially contains a stabilizer for the purpose of preventing the formation of peroxides in the solvent (2). The solvent was distilled just prior to extraction.

EXPERIMENTAL

Standardization of the Crude Drug.—Accurately weighed samples (10 Gm.) of the prepared ergot were assayed by the N. F. IX colorimetric method for the determination of total alkaloids (3). The procedure followed was identical to that of the N. F. with the exception of the final dilution of the acid extracts. In this procedure, rather than making one dilution representing 1 Gm. of crude drug per 100 cc. solution, a series of solutions of increasing concentrations, representing from 1 to 5 Gm. of crude drug per 100 cc. solution were prepared. The dilutions were modified in this fashion so that a curve could be established for comparison with the curves formed by similar dilutions of the extracts obtained by the use of tetrahydrofuran and its mixtures.

An ergonovine maleate calibration curve was prepared by making a series of solutions of U. S. P. Ergonovine Maleate Reference Standard in 0.10% tartaric acid solution. Aliquot portions of each solution were taken and allowed to react for thirty minutes with *p*-dimethylaminobenzaldehyde T. S. (3). The color intensities of these solutions were measured in a Klett-Summerson photoelectric colorimeter housing a No. 56 (green) filter (Fig. 1).

The colorimetric readings obtained from the assay of the crude drug by the N. F. IX method for the determination of total alkaloids was plotted on a

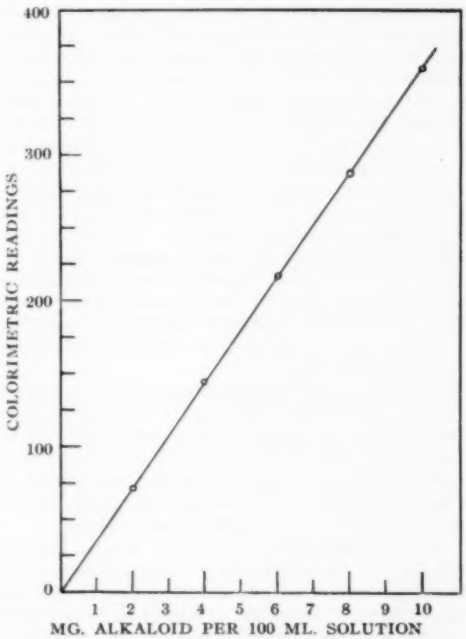

Fig. 1.—Ergonovine maleate calibration curve.

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Abstracted from a thesis presented to the Graduate Council of the University of Florida by Joseph M. Campo in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.
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graph (Fig. 3). When the reading of the 5-Gm. aliquot portion was referred to the reference curve, it was observed that the crude drug had an alkaloidal content of 7.8 mg. per 5-Gm. sample or 15.6 mg. per total 10-Gm. sample. This expression of total alkaloidal content and those of other assays in this paper are calculated in terms of Ergonovine Maleate Reference Standard.

Extraction with Tetrahydrofuran by Maceration.—Accurately weighed samples (10 Gm.) were macerated with 50 cc. of tetrahydrofuran for a period of forty-eight hours. At the end of the maceration period the supernatant tetrahydrofuran was decanted and the marc washed with a few small portions of fresh tetrahydrofuran to remove residual extract from the marc.

Tetrahydrofuran is miscible with water (2); consequently the extract cannot be applied to partition extraction with sulfuric acid solution as in the case of the official method in which chloroform and sulfuric acid solution are used. To overcome this difficulty, the tetrahydrofuran extract was evaporated to dryness by the use of vacuum without heat, applying the same procedure recommended in the official assay for the concentration of the chloroformic extract (3). The residue remaining upon evaporation of the tetrahydrofuran extract was then dissolved in 30 cc. of a mixture composed of nine parts of chloroform and one part of methanol containing 10% of stronger ammonia T. S. This solvent is identical with that recommended in the official procedure for the original extraction. Only by dissolving the residue in the same solvent as that of the official procedure could a comparable assay be established. The 30-cc. of extract thus obtained was treated in the same manner as in the official procedure with the exception of the final dilutions of the acid extracts which were modified in the same manner as in standardization of the crude drug. Reaction of these solutions with *p*-dimethylaminobenzaldehyde T. S. gave purple indole color reactions, indicating that alkaloids were extracted with nonalkaline tetrahydrofuran. The color intensities of these solutions (Fig. 3) when referred to the reference curve showed an alkaloidal extraction of 9.0 mg. per 10-Gm. sample or 57.8% extraction of total alkaloids.

Soxhlet Extraction.—Accurately weighed samples (10 Gm.) of the prepared ergot were macerated in a soxhlet extractor for one hour with enough tetrahydrofuran to moisten the drug. The sample was then extracted with 50 cc. of tetrahydrofuran adjusting the extraction to one cycle every ten minutes. Extraction was continued for a period of four hours. Assay of the extract showed an alkaloidal extraction of 9.4 mg. per 10-Gm. sample or 60.3% extraction of total alkaloids (Fig. 3).

Extraction with Tetrahydrofuran-Water Azeotrope (2) by Maceration.—Accurately weighed samples (10 Gm.) of the prepared ergot were macerated for forty-eight hours with 50 cc. of tetrahydrofuran-water azeotrope. Assay of the extract showed an alkaloidal extraction of 12.4 mg. per 10-Gm. sample or 79.4% extraction of total alkaloids (Fig. 3).

Soxhlet Extraction.—Accurately weighed samples (10 Gm.) of the prepared ergot were macerated in a Soxhlet extractor for one hour with enough tetrahydrofuran-water azeotrope to moisten the drug. The sample was then extracted with 50 cc. tetrahydrofuran-water azeotrope for a period of four hours. Total alkaloidal assay of this extract showed an alkaloidal extraction of 12.0 mg. per 10-Gm. sample or 76.9% extraction of total alkaloids (Fig. 3).

It was observed that the extracts obtained by both maceration and Soxhlet extraction with tetrahydrofuran-water azeotrope possessed a slight red color which was not observed in the extraction with tetrahydrofuran. This indicated extraction of the water-soluble color principles of the crude drug by the water present in the azeotrope. In both cases, however, the color was satisfactorily removed during the alkaloidal purification procedures of the assay.

Extraction with Tetrahydrofuran-Water Solution (50% V/V).—As noticed in the previous determinations, the presence of water in azeotropic combination with tetrahydrofuran has served to potentiate the alkaloidal extraction properties of the solvent. With this in mind, it was decided to extract the ergot with a tetrahydrofuran-water solution containing equal volumes of both solvents.

It is obvious that Soxhlet extraction cannot be used to measure the degree of alkaloidal extraction by using a 50% tetrahydrofuran-water solution, as the solvent which comes in contact with the crude drug would be the standard tetrahydrofuran-water azeotrope having a water composition of 4.3%. It was decided, therefore, that extraction of the ergot with this 50% solution must be restricted to maceration.

Accurately weighed samples (10 Gm.) of the prepared ergot were macerated with 50 cc. of a solution containing equal parts of tetrahydrofuran and water. The length of time of maceration was forty-eight hours as in the other determinations. The extract thus obtained had a deep red color, the intensity being much greater than that of the tetrahydrofuran-water azeotrope extract. This observation further helped to establish increased selectivity for

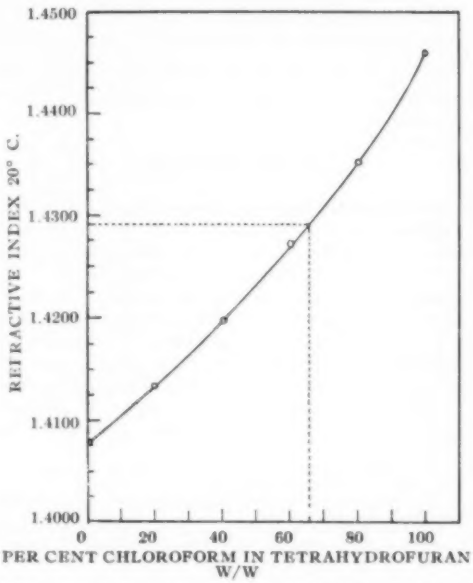

Fig. 2.—Refractive index of tetrahydrofuran-chloroform mixtures.

the color principles of the crude drug with increasing water content of the solvent. The color was removed during the purification procedures of the assay, which showed an alkaloidal extraction of 7.8 mg. per 10-Gm. sample or 50% extraction of total alkaloids (Fig. 3).

Preparation and Determination of Composition of Tetrahydrofuran-Chloroform Azeotrope.—This azeotrope was prepared by distilling a mixture of 200 cc. of tetrahydrofuran and 200 cc. of chloroform. The temperature gradually rose to 72.5° and remained at that temperature during the major course of the distillation. Only the constant boiling azeotrope (72.5°) was collected.

In order to determine the composition of the tetrahydrofuran-chloroform azeotrope a series of weight to weight solutions of chloroform in tetrahydrofuran were prepared and the refractive index of each solution determined by means of an Abbe refractometer at 20°. Using these data a refractive index curve was plotted (Fig. 2). The refractive index of the tetrahydrofuran-chloroform azeotrope taken at 20° was found to be 1.4292. Referring this reading to the refractive index curve revealed that the composition of the azeotrope was 65.5% chloroform in tetrahydrofuran (w/w).

Extraction with Tetrahydrofuran-Chloroform Azeotrope by Maceration.—Before considering the extraction procedure with the tetrahydrofuran-chloroform azeotrope, it would be best to point out first that this combination was found to be immiscible with water. It will be remembered that all combinations used up to this point have been miscible with water and consequently the necessary modification of the official procedure was necessary before these solvents could be applied to partition extraction. Complete evaporation to dryness is not necessary with this azeotrope because of its immiscibility with water, and the N. F. procedure may be more accurately employed.

Accurately weighed samples (10 Gm.) were extracted by the maceration procedure previously employed. Assay of the extracts showed an alkaloidal extraction of 14 4 mg. per 10-Gm. sample. The extent of alkaloidal extraction was thus 92.3% of total alkaloids (Fig. 3).

Soxhlet Extraction.—Assay of the extracts obtained by Soxhlet extraction of accurately weighed samples (10 Gm.) showed an alkaloidal extraction of 13.8 mg. per 10-Gm. sample or 88.5% extraction of total alkaloids (Fig. 3).

The extracts obtained by both maceration and Soxhlet extraction of the crude drug with tetrahydrofuran-chloroform azeotrope were very light in color, indicating a minimum of selectivity for the color principles of the crude drug. It was further observed that this azeotrope did not form emulsions as readily as with the other procedures employed during assay, indicating that the selectivity of this solvent for emulsion-forming constituents of the crude drug was very low.

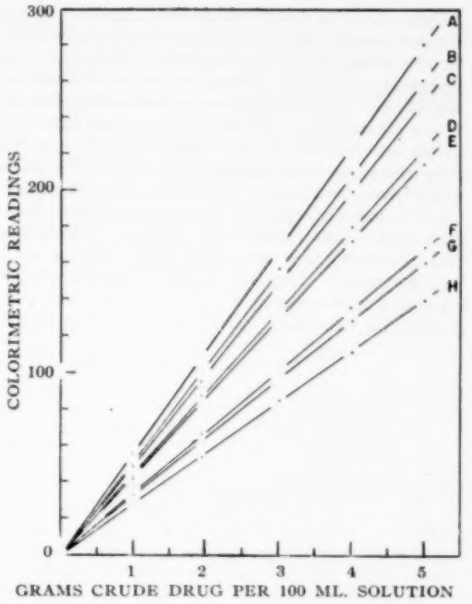

Fig. 3.—Extraction of ergot alkaloids.

A. N. F. IX assay. *B*. Extraction with tetrahydrofuran-chloroform (maceration). *C*. Extraction with tetrahydrofuran-chloroform (Soxhlet). *D*. Extraction with tetrahydrofuran-water (maceration). *E*. Extraction with tetrahydrofuran-water (Soxhlet). *F*. Extraction with tetrahydrofuran (Soxhlet). *G*. Extraction with tetrahydrofuran (maceration). *H*. Extraction with tetrahydrofuran-water 50/50 (maceration).

Assay of the Marcs for Residual Alkaloids.—The investigation up to this point has shown that each of the various solvent combinations employed has resulted in a specific degree of alkaloidal extraction. It became necessary therefore to determine whether this difference in the extent of alkaloidal extraction was due to selectivity for specific alkaloids or whether alkaloidal destruction was responsible for the

TABLE I.—SUMMARY OF RESULTS OBTAINED FROM EXTRACTION OF CRUDE ERGOT DRUG BY VARIOUS SOLVENTS

Mg. Alkaloid per 10-Gm. Sample	Solvent	Procedure	Mg. Alkaloids Extracted	Extraction, %
15.6	THF[a]	Maceration	9.0	57.8
15.6	THF	Soxhlet	9.4	60.3
15.6	THF-water azeotrope	Maceration	12.4	79.4
15.6	THF-water azeotrope	Soxhlet	12.0	76.9
15.6	THF-water (50% v/v)	Maceration	7.8	50.0
15.6	THF-$CHCl_3$ azeotrope	Maceration	14.4	92.3
15.6	THF-$CHCl_3$ azeotrope	Soxhlet	13.8	88.5

[a] Tetrahydrofuran.

difference. The marcs which were obtained by the various methods of extraction were dried and saved for this phase of the investigation. Each marc was assayed by the N. F. IX method or the determination of total alkaloids (3), to determine the presence of any alkaloid which might not have been extracted by the tetrahydrofuran mixtures. Very faint indole color reactions were obtained only from the marcs which were previously extracted with tetrahydrofuran alone and with tetrahydrofuran-chloroform azeotrope. Colorimetric determinations indicated that in each of these cases only 1 mg. of total alkaloids was found. Extracts obtained from the remaining marcs gave negative indole color reactions indicating that there were no alkaloids in these marcs. The data suggest that the differences in the degree of alkaloidal extraction is not due to specific selectivity, but rather to varying degrees of alkaloidal destruction.

Table I represents a summary of the data obtained by extraction of the crude drug by the various solvent combinations employed.

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Further Studies on the Metabolic Disposition of Dormison (3-Methyl-pentyne-ol-3) in Dogs and Man*

By PRESTON L. PERLMAN, DAVID SUTTER, and CAROL B. JOHNSON

A modified method for the determination of Dormison in biological materials is reported. The metabolic disposition of Dormison in dogs and in man was studied and the results of this investigation are presented.

IN A PREVIOUS publication (1) a series of animal experiments were described which had as their purpose the study of the modes of excretion and metabolic fate of the hypnotic carbinol, Dormison.[1] These experiments indicated that a major step in the metabolism of Dormison is the destruction of the ethinyl group.

Additional studies, in these laboratories, to extend the body of information on the metabolic disposition of Dormison have been carried out in dogs and in man and the results are the subject of this report.

The possible elimination of the drug as a conjugate was considered. Dormison, a tertiary alcohol, might be expected to conjugate with glucuronic acid. In addition, the role of the lungs and gastrointestinal tract as eliminative pathways have been examined.

* Received May 4, 1953, from the Biological Laboratories, Schering Corporation, Bloomfield, N. J.
[1] Registered trademark of Schering Corporation, Bloomfield, N. J.

METHODS

Dormison.—It was observed that the original method of analysis (1) yielded, on occasion, unaccountable high Dormison values when certain urine samples were analyzed. These elevated values occurred about the third to fourth day following drug administration, and appeared to be associated with the presence of an oily, black sediment which formed when ether extracts of the urine were reacted with the alkaline silver reagent. The sediment proved to be metallic silver which suggested the presence of an increased amount of reducing substance (*s*) in the urine. To circumvent the difficulty of analysis, a modification of the analytical procedure was introduced which embodied the basic principle of the original method, i. e., the reaction of the triple bond with 2 moles of silver. At the same time, the modification overcame the presence of interfering substances by using an aeration system for distilling the Dormison from the sample under analysis. The method as finally evolved follows.

An aliquot (75 ml.) of a twenty-four hour urine specimen or a fecal slurry sample is placed in a 250-ml. Pyrex centrifuge bottle fitted with a two-hole stopper and glass tubing as illustrated in Fig. 1. The bottle is placed in a hot water bath (80–85°) and aeration begun into a centrifuge bottle containing the alkaline silver reagent using a water aspirator system. A dilute sulfuric acid trap is installed between the alkaline silver reagent bottle and the aspirator to prevent escape of ammonia vapors into the laboratory. It was noted that the aeration would

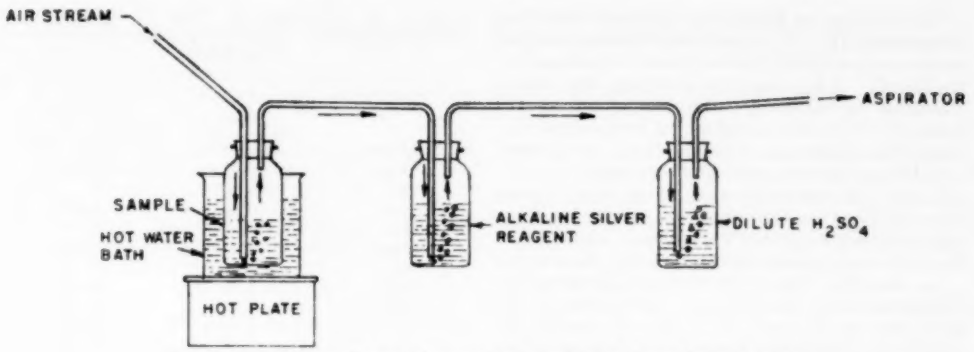

Fig. 1.—Diagram of setup for aeration method of Dormison analysis.

remove the ammonia from the silver reagent permitting formation of silver carbonate by the interaction of the CO_2 in the aeration stream with the silver nitrate. To prevent this, it is necessary to add 3 ml. concentrated NH_4OH to the silver reagent after one hour of aeration. With these precautions it was found that a two-hour aeration yielded satisfactory recovery (98–100%) of added Dormison.

This modified procedure results in a considerable saving in time by eliminating the necessity for a preliminary filtration or centrifugation of samples, and the time consuming double extraction with ether. It was found that with a train of aeration units connected to a manifold which in turn is connected to a single source of negative pressure one individual could run six samples simultaneously within a few hours.

The remainder of the method, after precipitation of Dormison with alkaline silver reagent, is the same as previously described (1).

Glucuronic Acid.—The method of Deichmann (2) was used.

RESULTS

Glucuronic Acid and Dormison Excretion in Dogs.—Glucuronide formation is considered to be a prevalent detoxification mechanism. Compounds which will conjugate with glucuronic acid are those possessing either hydroxyl or carboxyl groups. Dormison, being a tertiary alcohol, falls into the former category and might be expected to be eliminated as a glucuronic acid conjugate. For this reason, an investigation of this possibility was undertaken first in the dog and then in the human subject.

Normal, healthy beagles (male) were employed in the dog experiments. Capsules containing pure Dormison were administered at a dose of 300 mg./Kg. body weight.

In this experiment it was found that the Dormison excretion reached a peak at forty-eight hours but an additional four days were required to eliminate the remaining traces of drug. The overall recovery of Dormison in the urine was 1%, a figure slightly but not significantly lower than the figures reported previously (1).

The glucuronic acid level rose slowly to a peak at four days following drug administration. Control levels were resumed by about the tenth day after drug administration. Assuming that one mole of glucuronic acid conjugates with one mole of Dormison or its metabolite (*s*), it is possible to account for approximately 23% (Table I) of the administered dose as the glucuronide. Thus, a total of 24% of the administered drug could be accounted for as free drug and glucuronic acid conjugate.

The nature of the glucuronic acid conjugate is not yet elucidated although it would appear that the conjugate is not Dormison glucuronide but instead is a metabolite (*s*) of the original drug. This tentative conclusion is based on the fact that comparison of the Dormison drug content of a urine sample obtained from a dog receiving the drug before and after hydrolysis (6 *N* HCl for two hours at 85°) showed no difference in recoverable Dormison. Identical results were also obtained when urine from a dog receiving Dormison was subjected to the action of spleen glucuronidase (Viobin) at *p*H 4.5 (acetate buffer) for sixty hours and at 37°. If the Dormison were present as the glucuronide, hydrolysis of the glucuronide with either acid or glucuronidase treatment should have liberated at least some of the Dormison which would then have appeared as an increased urine Dormison content when compared to the unhydrolyzed sample. Efforts are being made to isolate and identify the glucuronide present in the urine of Dormison-treated dogs, and the results will be published at a later date.

TABLE I.—GLUCURONIC ACID EXCRETION BY DOGS FOLLOWING DORMISON ADMINISTRATION

Animal No.	Oral Dose (Gm.) Dormison	Total Glucuronic Acid (Gm.) Excreted Above Base Level[a]	Per Cent Dormison Excreted as Glucuronic Acid Conjugate (Calc.)
4	12.96	6.92	27
8	7.44	2.49	17
4	6.75	3.14	24
9	5.25	2.84	27
9	2.45	0.92	19
		Average	22.8

[a] Base level of glucuronic acid for dogs in this laboratory averaged 27 mg./day with a range of 14–34 mg./day. The base level is the average daily excretion for the three days immediately preceding drug administration.

The Lungs as an Eliminative Pathway in the Dog (Experiment II).—One experiment was done in a dog to determine whether any Dormison is eliminated via the breath. A dog was given a 200 mg./Kg. dose of Dormison by capsule and thirty minutes later administered sufficient pentobarbital intravenously to induce the surgical plane of anesthesia. A tracheal cannula was put into place and connected to a two-way valve of such design that all the expired gases from the lungs were directed to a vessel containing alkaline silver reagent. This reagent served effectively to trap any exhaled Dormison as the insoluble silver complex. Up to seven hours following the administration of the Dormison no evidence of the drug could be detected in the expired gases which would indicate that the drug does not appear in the lung gases to an appreciable extent during and immediately following the hypnotic phase of the drug.

Feces.—In order to arrive at an excretion balance for the drug, and also to ascertain the importance of the gastrointestinal system as an eliminative pathway, stool analyses were carried out on several dogs following oral administration of 200 mg./Kg. Dormison. The findings were negative for all samples. It is realized, however, that the flora in the gastrointestinal tract might be able to render the ethinyl group nonreactive in a manner similar to that already found (1) with certain tissue slices.

Dormison and Glucuronide Excretion by Normal Human Subjects.—Previously, data (3) obtained on urine Dormison levels in normal human subjects ingesting a single 100-mg. dose of Dormison were presented. The results showed no demonstrable drug in the urine following this dose. It was deemed of importance to extend our studies to higher dose levels and also to measure urinary glucuronic acid as was already done in the dog. It was possible to arrange for the collection of daily urines from normal human subjects receiving single doses of Dormison at a level of 500, 1,000, and 3,000 mg. of the drug.[2]

Following the administration of the drug, the Dormison level in the urine reached a peak of 2–3% of administered dose during the first forty-eight hour collection periods after which the level steadily fell. A total of 4.3–11.4% of the administered dose was found in the urine following the 500 and 1,000-mg. dose and 6.9–14.2% from the 3,000-mg. dose.

Examination of the glucuronic acid levels of human subjects at the various drug doses revealed no significant responses which could be attributed to the drug and for this reason a calculation of the per cent Dormison conjugated with glucuronic acid is not included in Table II.

[2] Through the courtesy and assistance of Drs. G. Babcock and G. Kenneth Hawkins of our Clinical Research Division.

TABLE II.—GLUCURONIC ACID EXCRETION BY HUMAN SUBJECTS FOLLOWING VARIOUS DOSES OF DORMISON

Subject	Oral Dose (Gm.) Dormison	Total Glucuronic Acid (Gm.) Excreted Above Base Level[a]
Am.	0.5	0.04
De.	0.5	0
Ke.	0.5	0.16
Ri.	0.5	0
Am.	1.0	0.32
De.	1.0	0.41
Ke.	1.0	0.30
Ri.	1.0	0.26
Si.	3.0	0.05
Hy.	3.0	0.35
Ba.	3.0	0.23

[a] Base level of glucuronic acid for the human subjects averaged 187 mg./day with a range of 115–332 mg./day.

SUMMARY AND CONCLUSIONS

1. A modified method for the determination of Dormison in biological materials is reported.

2. The effects of single oral doses (300 mg./Kg.) of Dormison in dogs are presented with reference to urine Dormison excretion and glucuronic acid excretion. The data indicate that 17–27 per cent of ingested drug is eliminated as a metabolite (*s*) conjugated with glucuronic acid. Approximately 1 per cent of the ingested drug appeared as an unchanged compound in the urine.

3. No evidence for fecal elimination of Dormison was found in the dog.

4. No evidence for elimination of the drug via the lungs was found in the dog.

5. A single dose of 500, 1,000 and 3,000 mg. was administered to normal human subjects and urine Dormison and glucuronic acid levels were measured. Up to 10 per cent of the drug was eliminated unchanged and only equivocal amounts were eliminated as a glucuronic acid conjugate.

6. In man, even following relatively large doses, efficient alteration of the drug occurs.

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The Alkaloid of *Banisteriopsis inebrians* Morton*

By FRANK D. O'CONNELL† and E. V. LYNN‡

The stems of *Banisteriopsis inebrians* were found to contain 0.15 per cent of the alkaloid harmine, but the other alkaloids that accompany harmine in *peganum harmala* could not be detected. The leaves were found to contain an alkaloid which was partly identified as harmine.

BANISTERIOPSIS INEBRIANS (*Malpighiaceae*) was first described by Morton in 1931 as a tall liane, climbing more than 300 meters. The branches are terete and smooth, with thorns up to 4 millimeters in diameter, and bearing numerous, brown, glabrous lenticels. The leaves are broadely elliptical, olive-green in color, and glabrous except for a few persistent hairs (1).

In some South American countries the natives use in certain ceremonial rites an indefinite mixture of plants that they call "yage." According to Holmes, Bayon extracted from one of these mixtures an alkaloid that he named telepathine because of the physiological action of yage (2). Later, Villalba examined a plant called yage and separated from it two alkaloids that he named yageine and yagenine (3).

In 1924 Rusby identified a plant called yage in the Amazon Basin as *Banisteria caapi* Spruce (4), since then renamed by Morton as of the genus *Banisteriopsis* (5). Seil and Putt extracted from Rusby's samples three alkaloids, but did not investigate their properties (6). In 1928 Merck isolated the principal alkaloid and named it banisterine (7), but subsequent investigators have identified it as harmine (8, 9), previously obtained from the seeds of *Peganum harmala* L. (10). It has not been definitely established that *B. caapi* contains more than one alkaloid, nor that yageine and telepathine were from this plant, although both of the last names, as well as banisterine, are commonly given as synonyms of harmine. Yagenine has not been confirmed, nor have the second and third alkaloids of Seil and Putt.

EXPERIMENTAL

The material used in this study was received by Heber W. Youngken of the Massachusetts College of Pharmacy from Columbia, South America. It consisted of the stems and the leaves of *Banisteriopsis inebrians* Morton, as identified by him and confirmed by R. E. Schultes of the Bureau of Plant Industry, Washington, D. C.

The total amount of alkaloids was estimated in the coarsely ground stems, using the method described for belladonna leaf U. S. P. XIV. The value found was 0.145%, calculated as harmine $C_{13}H_{12}N_2O$.

Isolation of Alkaloids.—Approximately 700 Gm. of the coarsely ground stems was moistened with a mixture of 1 part of stronger ammonia water, 1.25 parts of alcohol, and 2.5 parts of ether. The moistened material was packed into a large Soxhlet apparatus and allowed to macerate for twelve hours, after which time it was exhausted by percolation with chloroform. The chloroformic solution was completely extracted with normal sulfuric acid. After filtration, the acid solution was heated to boiling and rendered alkaline with stronger ammonia water, giving a fine brown precipitate. This precipitate was filtered, dried, redissolved in chloroform, again extracted with acid, and precipitated with ammonia water. After heating the mixture, the precipitate assumed the form of conglomerates of brown, needle-shaped crystals, which were removed by filtration and dried.

Purification.—The dried precipitate was dissolved in a minimum amount of hot methanol, mixed with activated charcoal, and heated for several minutes on a water bath. The hot mixture was filtered, and the filtrate upon standing deposited white, shiny crystals which were filtered, washed, and dried at room temperature. The melting point of 260° (corr.) with some decomposition was not altered by recrystallization. The compound was soluble in alcohol, methanol, chloroform, or acids, but was only slightly soluble in water or alkalies.

The Hydrochloride.—A portion of the alkaloid was dissolved in alcohol, and to this solution was added ether acidified with hydrochloric acid. The hydrochloride precipitated from the mixture in the form of white crystals. It gave a blue fluorescence under ultraviolet light and melted at 261° with decomposition. In the anhydrous state it melted at 319° and contained about 12.5% of chlorine.

Identification.—The identity of the alkaloid as harmine was determined by comparison with a carefully purified sample from the seeds of *Peganum harmala* L. Both gave the same spectrogram, with minimum absorption at 272 mμ and maximum absorption at 300 mμ. Both melted at about 260° with decomposition, and a 50% mixture of the two melted at the same temperature. The hydrochlorides were both colorless but gave a blue fluorescence under ultraviolet light.

Other Alkaloids.—No evidence could be obtained for the presence of any other alkaloid. Harmaline, which can be detected by Amelink's acetaldehyde test (11) in the crude product from *Peganum harmala*, was not present in the precipitated alkaloids

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† Abstracted from a thesis presented to the Graduate Council of the Massachusetts College of Pharmacy by Frank D. O'Connell in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.
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of *B. inebrians*. Harmalol is easily demonstrated in harmala by the green fluorescence under ultraviolet light of an aqueous solution, but no such evidence could be found for its occurrence in *B. inebrians*.

The Leaves.—The leaves were extracted in a manner similar to the one used for the stems. A solution of the resulting product in hydrochloric acid gave a precipitate with Mayer's reagent and a blue fluorescence under ultraviolet light. In view of these facts, it may be assumed that harmine is also present in the leaves.

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Notes

A Note on the Use of Amberlite IR-45 in the Ion Exchange of Sympathomimetic Amines*

By MURIEL C. VINCENT, EDWARD KRUPSKI, and LOUIS FISCHER

WORK IN THIS laboratory[1] on the determination of sympathomimetic amines by means of ion exchange has shown that the various methods reported in the literature for amines (1–3) were not satisfactory with all of our compounds. A number of ion exchange resins were used experimentally by the authors. Amberlite IR-45 was found to be suitable for the quantitative separation of a large group of sympathomimetic amines including several which have phenolic groups and could not be satisfactorily separated with other ion exchange resins.

EXPERIMENTAL

Procedure.—Eight grams of the anion exchange resin, Amberlite IR-45, were soaked in 25 ml. of distilled water for twenty-four hours. With the aid of additional distilled water the mixture was poured into a chromatographic column, 2 cm. x 20 cm., in which a small piece of glass wool had previously been placed. Another small piece of glass wool was placed on the top of the column. The resin was regenerated before each determination by passing 50 ml. of 4% sodium carbonate solution through the column. The column was washed free of sodium carbonate with 200 ml. of previously boiled and cooled distilled water.

Twenty-five to 50 mg. of amine salt was dissolved in 25 ml. of 50% ethyl alcohol, the solution was passed through the column, and the filtrate was collected and saved. The column was then rinsed with an appropriate amount of 75% ethyl alcohol to remove the amine base, and the rinsings were added to the filtrate. The combined solution was titrated with 0.1 *N* hydrochloric acid using a *p*H meter or 2 drops of methyl red T. S. as indicator. The results are recorded in Table I.

TABLE I.—RECOVERY OF SYMPATHOMIMETIC AMINES

Amine Salt	75% EtOH Required to Remove Free Amine from Column, Ml.	Recovery, %
l-Ephedrine sulfate	200	100.05, 98.85, 98.55, 98.26
Racephedrine sulfate	200	99.93, 99.61, 97.45
Propadrine hydrochloride	100	98.94, 98.16
Paredrine hydrobromide	200	98.43, 98.23
Aramine hydrogen tartrate	200	99.14, 98.67, 98.24, 97.20
Orthoxine hydrochloride	200	99.99, 99.94, 98.95
Privine hydrochloride	200	99.85, 99.60, 99.60
Vonedrine hydrochloride	200	100.06, 99.03, 98.27
dl-Methamphetamine hydrochloride	200	99.81, 99.75, 99.00
d-Methamphetamine hydrochloride	200	100.56, 99.62, 99.36
Tuamine sulfate	200	98.47, 97.20

Details of this and further work in the ion exchange and separation of sympathomimetic amines will be published at a later date.

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* Received July 22, 1953, from the College of Pharmacy, University of Washington, Seattle, Wash.
[1] Pharmaceutical Chemistry Laboratory.

A Note on the Optical Crystallographic Properties of Certain of the Cardiac Glycosides*

By JOHN W. SHELL† and NORMAN F. WITT‡

The optical crystallographic properties of some of the most important cardiac glycosides have been determined. These properties afford a rapid and accurate means for their identification, as well as a basis for future molecular orientation studies.

FREQUENTLY in pharmacologic research with the cardiac glycosides it is necessary to detect very small amounts of these compounds. Inasmuch as the optical crystallographic properties of a compound are specific, are rapidly determined, and require only small amounts of material, investigations of these properties of some of the more important members of the cardiac glycoside group have been made in this laboratory.

These properties serve not only as a positive means for identification, but also as an aid for future molecular orientation studies.

EXPERIMENTAL

The compounds used were recrystallized from ethyl alcohol solution, and the melting point of each was checked and reported. The optical properties were determined by means of a petrographic microscope and light source as described by Biles, Witt, and Poe (1). The immersion method and Becke test was used for refractive index determination. The immersion media employed were prepared according to Chamot and Mason (2) and Larsen and Berman (3). The refractive indexes of the liquids so prepared were calibrated at 20° and 25° by use of the Abbe and Pulfrich refractometers. In this manner, a series of liquids was obtained which covered the range of refractive indexes from 1.397 to 1.815 by differences of 0.004 to 0.005. The accuracy of the refractive indexes reported is ±0.002.

The orientations of the crystals were determined by use of interference figures, centered to give principal refractive indexes as follows:

Acute bisectrix	+	α and β
	−	β and γ
Obtuse bisectrix	+	β and γ
	−	α and β
Optic normal	+ or −	α and γ
Optic axis	+ or −	β

The interference figures served also for dispersion observation and aided in the determination of the optical orientation of each compound.

A Leitz universal stage with five axes of rotation was used extensively in this investigation for measurements of the optic axial angle and for accurate determination of optical orientation.

SUMMARY

1. The optical crystallographic properties of digitoxin, digoxin, ouabain, and lanatoside-C have been determined.

2. These properties afford a means for rapid and specific identification using only small amounts of the compounds.

TABLE I.—THE OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF DIGITOXIN, DIGOXIN, OUABAIN, AND LANATOSIDE-C

Compound	Melting Point, °C.	Crystal Habit	Crystal System	Optic Orientation	Optic Sign	Optic Axial Angle	Axial Dispersion	Refractive Indexes: Alpha	Beta	Gamma	Common Orientation
Digitoxin	254	tabular	monoclinic	Y = B ZΛA = 40°	+	35°[a]	V > R	1.513	1.518	1.568	Optic normal
Digoxin	234	tabular	monoclinic	Y = B ZΛC = 20°	−	67°	V > R	1.466[a]	1.553	1.593	Inclined optic axis
Ouabain	187	acicular	orthorhombic	Y = B Z = C	+	31°	None	1.533[a]	1.536	1.568	Inclined obtuse bisectrix
Lanatoside-C	255	tabular	monoclinic	Y = B XΛC = 27°	+	71°[a]	R > V	1.502	1.524	1.568	Inclined optic axis

[a] Calculated.

* Received March 24, 1953, from the Chemical Microscopy Laboratory, University of Colorado, Boulder.

† Graduate Student, University of Colorado.

‡ Professor of Chemistry and Chairman of the Department of Chemistry.

The authors are grateful to Eli Lilly and Company for samples of digitoxin and ouabain, to Burroughs Wellcome Company for a sample of digoxin, and to Sandoz Chemical Company for a supply of lanatoside-C.

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1954 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1954 Chilean Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented each year at the annual meeting of the ASSOCIATION.

Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1954 Award, nominations must be received on or before January 1, 1954.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION'S Council. The present committee includes Justin L. Powers, chairman; Paul Block, Louis Gershenfeld, Harvey B. Haag, Lloyd M. Parks, Heber W. Youngken, and Charles O. Wilson.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. Travel expense to the meeting at which the award is to be made is provided for up to $250.

The award is now in its sixth year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist; the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, and the third was Dr. C. P. Leblond, Professor of Anatomy, McGill University, Montreal, Canada. The fourth award was shared jointly by Dr. George Moore of the University of Minnesota Medical School and Dr. Moses Ashkenazy, Chief of Neurosurgery, Veterans Hospital, Houston, Tex. Dr. Vernon H. Wallingford, Mallinckrodt Chemical Works, St. Louis, Missouri, was chosen as the recipient of the fifth award which was conferred during the 1953 Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Book Notices

Quantitative Pharmaceutical Chemistry. By GLENN L. JENKINS, JOHN E. CHRISTIAN, and GEORGE P. HAGER. 4th ed. McGraw-Hill Book Co., Inc., New York, 1953. x + 534 pp. 15 x 21.5 cm. Price $6.50.

This unique textbook represents the fourth edition, the first of which was published more than seventeen years ago. During these years it has proved to be an unusually effective textbook for students of pharmacy and a valuable reference book for analysts working with pharmaceutical products. The book is so well and favorably known among pharmaceutical educators that little need be said concerning it. In the fourth edition the authors have followed the design of the previous editions, but the theory and the 126 typical procedures and 75 tables have been thoroughly revised to conform to the official changes made in U. S. P. XIV and N. F. IX. Many new and additional questions and problems have been added to permit greater selection for assignment by the instructor. The authors have also included much new material pertinent to instrumental analysis and the book is replete with illustrations many of which are entirely new.

The authors are to be congratulated upon the judgment they have shown in accomplishing a third revision which has resulted in many notable improvements over previous editions.

The Use of Drugs—A Textbook of Pharmacology and Therapeutics for Nurses. By WALTER MODELL, M.D., and DORIS J. PLACE, R.N. Springer Publishing Co., Inc., New York, 1953. 468 pp. 14 x 22 cm. Price $4.50.

In this textbook, written especially for the nurse, scientific data have been avoided and the principles of pharmacology and therapeutics are presented simply.

Color in Business, Science, and Industry. By DEANE B. JUDD. John Wiley & Sons, Inc., New York, 1952. 401 pp. Illustrated. 15.5 x 24 cm. Price $6.50.

This book by Deane B. Judd, Chief of the Colorimetry Unit of the National Bureau of Standards, Washington, D.C., constitutes an attempt to answer questions asked by thousands of men in commerce and industry during the past 20 years. The author has succeeded in striking a balance between a highly scientific presentation and a popular treatise on the difficult subject of color as it is used in business and industry.

The book is divided into three parts, in the first of which basic facts are presented relating to the eye, and basic terms employed in color nomenclature are defined. Color matching and color deficiencies are also discussed. Part II is devoted to tools and techniques, while Part III discusses the physics and psychophysics of colorant layers. The book is also provided with an appendix including pertinent references and an excellent index. Among the many subjects discussed are the appropriate use of the spectrophotometer, the appropriate use of a photoelectric colorimeter, and the appropriate use of both. The limitations of the GE recording spectrophotometer and the Beckman spectrophotometer are treated adequately, and the practical value of the Munsell and the Ostwald color systems are explained. It would appear that the author has succeeded admirably in his attempt to present visual psychophysics of color in terms that are practical and useful.

The Formulary of the Nebraska State Medical Association. 1st ed. Nebraska State Medical Association, 1315 Sharp Building, Lincoln, Nebr., 1953. 90 pp. Paper bound. 13 x 19 cm. Price $1.50.

This little book was prepared under the auspices of the joint Committee on Pharmacy and Therapeutics of the Lincoln Hospitals and the College of Pharmacy of the University of Nebraska. According to the preface, ". . . an effort has been made to include drugs of proven therapeutic usefulness. Duplication of products under various trade names and needless combinations are avoided. Excessively costly preparations have been reduced to a minimum consistent with good therapeutics. The need of many combinations may be met by ordering individual doses of the components. For example, aminophylline and phenobarbital combinations may be obtained by ordering the two separate drugs.

"The drugs and preparations included in this formulary fall into four categories. Those official in the Pharmacopeia of the United States, Fourteenth Revision; the National Formulary, Ninth Edition; New and Nonofficial Remedies, 1952 edition, and a selected list of drugs and preparations not recognized by the preceding compendia, but selected because of their therapeutic value. The use of trade names in this formulary does not constitute an endorsement, but is only for identification."

A Manual for the Organic Chemistry Laboratory. By LEIGH C. ANDERSON and WERNER E. BACHMANN. John Wiley and Sons, Inc., New York, 1953. xvi + 164 pp. 23 x 28 cm. Paper and ring bound. Price $2.75.

This competently written manual is designed for the beginning student in organic chemistry, regardless of his specialized requirements and contemplated training. Preparative methods and general techniques are described in a series of 60 experiments including the synthesis of approximately 50 organic compounds, about equally divided between aliphatic and aromatic chemicals. The compounds chosen for syntheses are intended to illustrate principles and to familiarize the student with basic methods of synthesis of wide general application.

Most of the experiments have been used for many years in the courses of beginning organic chemistry in the laboratories of the University of Michigan. They have thus been carefully checked by faculty and students—a feature which induces confidence in the character of the manual.

A Manual for the Organic Chemistry Laboratory is bound in durable paper covers by means of spiral rings. It can be recommended to all students who are required to complete a laboratory course in organic chemistry.

Soap Manufacture. Vol. I (in 2 volumes). By J. DAVIDSOHN, E. J. BETTER, and A. DAVIDSOHN. Interscience Publishers, Inc., New York, 1953. (One in a series of monographs on the chemistry and technology of fats, oils, and related compounds.) xii + 535 pp. 16 x 23.5 cm. $12.50.

Soap Manufacture is one of the latest in a series of monographs on fats and oils published by Interscience Publishers. The book is designed to fill the need of a comprehensive treatise covering advances in the science and practice of soap making. The book is divided into four parts and includes a total of 22 chapters. In the first chapter history and statistics of the soap industry are presented, followed by five chapters devoted to the theoretical principles that require understanding as a foundation for studying the practical soap processes which are described in the subsequent chapters. Other chapters of this volume deal with the raw materials used in the industry. Part *B* deals with inorganic and organic soap builders and fillers, and with the fatty raw materials. In Part *C* practical soap-boiling processes are described and discussed, and in Part *D* special soap products including medicated soaps are covered.

The second volume, when published, will be devoted to a description of equipment, design, and operations conducted on the soap-boiling floor and in those departments in which soap is converted into its final shape. The book is documented throughout, is provided with an adequate index, and the printing and binding are of good quality.

Major Metabolic Fuels. Brookhaven Symposia in Biology No. 5, September, 1952. Brookhaven National Laboratory, Upton, New York. (Associated Universities, Inc., under contract with the U. S. Atomic Energy Commission.) 234 pp. Paper bound. 18 x 25.5 cm. (Copies can be obtained from the Office of Technical Services, Department of Commerce, Washington 25, D.C.).

This publication records a symposium held September 3–5, 1952, by the Biology Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, New York. Associated Universities operates under contract with the United States Atomic Energy Commission.

The previous numbers of this series have been devoted to biological applications of nuclear physics, radioiodine, assimilation reactions in biological systems, and chemistry and physiology of the nucleus. The papers in this volume are arranged in the order in which they appeared in the Symposium program and, as the title suggests, several of the papers are devoted to the mechanics involved in the utilization of glucose and its derivatives, to protein as a metabolic fuel, and to the metabolism of fats, including the biosynthesis of fatty acids in cell-free systems.

The book is provided with an index and each paper is well documented with references to the original literature on the varied subjects covered.

Antibiotics. 2nd ed. By ROBERTSON PRATT and JEAN DUFRENOY. J. B. Lippincott Co., Philadelphia, 1953. xv + 398 pp. 16 x 24 cm. Illustrated. Price $7.50.

The first edition of this book was published in 1949. Rapid advances in the field of antibiotics have made it necessary to revise the first edition thoroughly and to add much additional material. The first edition was reviewed in THIS JOURNAL, **39**, 113(1950). The aim of the book is to present in a succinct, integrated plan the facts and principles of fundamental and permanent value relating to antibiotics. During the course of the revision, five new chapters have been written, and to show each new phase of the subject of antibiotics in its proper perspective, this edition has been organized into four parts covering fundamental aspects, industrial aspects, applied aspects, and modification of biologic and social systems. At the end of each chapter a few guides to the pertinent literature are listed. The style employed in the book is to be commended because of the interest it stimulates and its easy readability. The book is well illustrated, provided with an excellent index, and the printing and binding are of good quality. It can be recommended especially for those with scientific training who wish to acquire a general knowledge of the entire antibiotic field as it exists today.

Organic Syntheses. Vol. 33. CHARLES C. PRICE, Editor-in-Chief. John Wiley & Sons, Inc., New York, 1953. 115 pp. 15.5 x 23.7 cm. Price $3.50.

The design, aim, and function of *Organic Syntheses* are well known to all organic chemists. This 33rd volume presents procedures for the synthesis of some 40 compounds including the following: atrolactic acid; benzhydryl *beta*-chloroethyl ether; benzoguanamine; butyrchloral; creosol; 2,2-dimethylpyrrolidine; ethyl *alpha*-(1-Pyrrolidyl)-propionate; furfural diacetate; 3-methylcoumarone; 1,4-naphthoquinone; nicotinonitrile; *m*-nitrobenzazide; *o*-nitrocinnamaldehyde; (*o*-nitroveratraldehyde; *alpha*-phenylcinnamic acid; 4-phenyl-*m*-dioxane; pyridine-N-oxide; 2-(1-pyrrolidyl)-propanol; 3-thenaldehyde; 3-thenoic acid, and 3-thenyl bromide.

Diuretic Therapy—The Pharmacology of Diuretic Agents and the Clinical Management of the Edematous Patient. By ALFRED VOGL, M.D. The Williams & Wilkins Co., Baltimore, 1953. xiii + 248 pp. 16 x 23.5 cm. Price $5.

The author has assembled all of the pertinent material relating to diuretic therapy in this book. It presents in a concise manner a comprehensive knowledge of diuretic therapy, of special interest to the physician, the internist, the cardiologist, and the general practitioner. It shows physicians how to adapt diuretic treatment to the individual needs of each patient.

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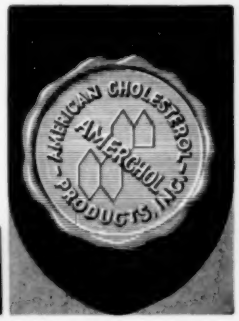

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